THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME 151

BALTIMORE, MD.
1947

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REGIONAL INTRATHORACIC PRESSURES AND THEIR BEARING ON CALCULATION OF EFFECTIVE VENOUS PRESSURES[1]

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Received for publication July 28, 1947

The registration of intrathoracic pressure with reasonable accuracy concerns many fields of investigations. It has been a major problem for us in making the best available estimates of effective venous pressure. The principle enunciated by Henderson and Barringer (1) that effective filling pressure is not determinable by manometers equilibrated with atmospheric pressure, but that it must be calculated as the algebraic difference between pressure within and around the atria is unquestionably sound. It has become increasingly apparent, however, that owing to the complexity of pressure variations within and around the atrium certain refinements are required in the application of the principle. These concern evaluation of the two components—atrial or central venous pressure and extracardiac pressure. The evaluation of the former has been discussed on previous occasions (2, 3). This report is concerned chiefly with the question as to how pressures around the heart can be most satisfactorily and expeditiously determined or estimated.

On the assumption that the elastic retraction forces of the lungs are evenly distributed within the thoracic cavity it has been common practice to equilibrate venous with intrapleural pressures. We have long recognized the possibility that with procedures commonly employed, technical errors may unwittingly enter which invalidate the measurement of intrathoracic pressure (3). We also were not satisfied that intrapleural pressures are comparable to those existing around the heart and that they change directionally with them. Brookhart and Boyd (4) have recently investigated this question. They recorded atrial pressures differentially against pressures in air pockets or small balloons placed in various regions of the chest. They also recorded pressures in several different pockets differentially.

Their meticulous studies seem to demonstrate *1*, that the pressure in a lax balloon around the heart is higher than in pleural air pockets; *2*, that the differ-

[1] Supported by a grant from the Commonwealth Fund.

ence is accentuated during inspiration, and *3*, that the difference is diminished or abolished when a partial pneumothorax is produced. The higher extracardiac pressure was attributed to distortion of lung tissue by the heart thereby reducing its elastic pull. On the basis of such findings the authors properly question whether true effective venous pressures can be calculated by the difference between right atrial pressure and that in a lateral pleural pocket.

This investigation, carried out during the spring of 1946, was concerned with the problems of determining *1*, the natural pressures which exist in various regions of the chest; *2*, the forces concerned in producing regional pressure differences; *3*, the most favorable region for recording pressures which enable one to follow trends of effective venous pressure, and *4*, evaluation of the validity of conclusions previously drawn regarding changes in effective venous pressure during shock and hemorrhage.

Experiences previous to this study. During the past six years many tests were made to assess the relative merits of different procedures. From these, certain principles evolved which eventually determined the choice of method used in this study. Since these principles are also involved in comparing our results with those of others it seems advisable to recount them briefly.

1. Perhaps the most common procedure for measuring intrathoracic pressure consists in inserting a tube or trocar connected to a water manometer or a calibrated tambour through an intercostal space. Separation of the pleural layers is then accomplished by admitting a small volume of air, purposely or surreptitiously. If a water manometer is used a definite volume of air enters mechanically, the amount depending on the diameter of the manometer tubes and the intrathoracic pressure. Since intrathoracic pressure varies during inspiration and expiration air passes to and fro, in this way varying the size of the air pocket. Pressures recorded from such air pockets are obviously higher than intrapleural pressure, the magnitude of the difference depending on the size of the air pocket (5). Unfortunately, air pockets so created tend to alter in size owing to absorption of air, surreptitious leakage, or shifts of air between pleural layers. When this occurs pressure readings alter, while true intrapleural pressure may remain the same. Air pockets of a similar nature may be created around cannulae thrust deeply into the chest between pulmonary lobes until they come to lie between pleural and pericardial layers. Air from such pockets tends to be dispersed even more quickly by movements of the heart; consequently, pressure readings become very erratic.

2. In early studies of effective venous pressures one of us (6) deluded himself with the belief that differential pressures could be recorded from the right atrium and intrapleural cavities by connecting these to two limbs of a water manometer. In subsequent tests a differential optical manometer was substituted. In the latter, pressures could be recorded either separately or differentially. It was found that such differential curves frequently conceal important details of pressure changes and fail to detect accidental errors which are obvious at once when pressures are recorded separately. For example, if an atrial cannula accidentally becomes occluded by a clot or by a shift in its position, a differential curve

continues to be inscribed, but it is of course meaningless. We have had occasion to detect similar unnoticed errors in registration of differential pressures from several regions of the thorax owing to temporary displacement of cannulae through a deep breath. We therefore consider it safer to record each pressure directly rather than differentially.

3. In order to avoid variations in size of air pockets previously mentioned we attempted to replace them with small rubber balloons filled with measured quantities of air or liquid. In our hands they yielded only unsatisfactory results, particularly when placed in extra-cardiac regions. In the first place their introduction requires opening and subsequent closure of the chest. It is highly questionable from our experience whether natural pressure relations can be recreated in all localities of the thorax after its closure. It is almost impossible to remove all the air between pleural surfaces either through use of slow suction or by expansion of lungs under positive pressure. We suspect that even more air may be trapped between pleural surfaces and the pericardium. Unless such pockets are excluded in an experiment the pressures recorded from such areas by rubber balloons automatically become higher than under normal conditions. In the second place, partially filled balloons must be used in order to obviate elastic forces of the rubber. According to Brookhart and Boyd (4), they should be flexible enough to adapt themselves to the contours of the heart and lungs. When such balloons are distended with small measured volumes of air only a part of the volume introduced can be withdrawn after closure of the chest, indicating that their cavity becomes divided or that the membrane occludes the cannula openings. Under the influence of the heart beat the balloon cavity may similarly be divided at critical times, in which event one registers from balloon pockets of varying capacity. Finally, pressure variations are recorded from balloons inserted between the heart and lungs even when the chest is opened. Impacts from the beating heart which produces pressure changes are unavoidable. It is improbable that these difficulties can be overcome by the special technique employed by Brookhart and Boyd. Unfortunately, records of pressure changes which actually occurred in their rubber balloons were not published. Such records might have proved or disproved their claims that pressures between the lateral aspects of the pericardium and adjacent lungs were faithfully recorded. On the other hand, artifacts that might have been present were apparently obscured in differential records taken, but some of these (e.g., their fig. 7) indicate that the effects of cardiac impulses were not entirely eliminated. At any event it seemed important to confirm their conclusions by other techniques.

4. We have found that intrathoracic pressure can be recorded by calibrated optical capsules through air systems without the introduction of more than very insignificant quantities of air. An intrapleural space can be created mechanically by pushing the visceral pleura and lungs inwards by a suitable thoracic cannula. The air which enters is limited by the slight depression of the rubber diaphragm of the segment capsule; it is certainly less than 1 cc. Similar airless pockets can be created mechanically in regions near the heart, as Dr. Werle demonstrated in 1942. In fact, our first conclusions regarding changes of effec-

tive venous pressure during hemorrhage and shock (2) were based on intrathoracic pressures recorded from such pockets near the heart. Difficulty was experienced, however, in maintaining patency of such artificial cavities in deeper regions over long periods of time required in studies on shock. Hence, in most of our subsequent studies we resorted to registration of pressures from the right lower pleural cavity with the technique outlined above. Admittedly, the forceful creation of such cavities also develops small tensions which cause recorded pressures to deviate slightly from actual pressures; but it is likely to cause less distortion than insertion of balloons and obviates opening of the chest with its consequences.

5. We have also tried the expedient of studying effective venous pressures during shock and hemorrhage in animals with an induced partial pneumothorax which equalizes pressures throughout the thoracic cavity. While a few gratifying experiments have thus been realized, two difficulties tend to complicate the course of events: *1*, the pneumothorax may extend to the opposite side at an inopportune time; *2*, as a result of reduced lung inflation the hyperpnea which follows a massive hemorrhage becomes greatly intensified and interferes with satisfactory registration of cardiovascular pressure changes. The possible induction of anoxemic hypoxia must also be conceded.

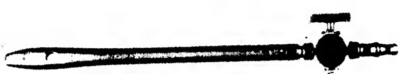

FIG. 1

PROCEDURE. In this investigation pressures were recorded from various regions of the thorax according to the principle outlined above, i.e., by mechanical creation of artificial cavities without introduction of more than insignificant volumes of air. A thoracic cannula designed many years ago for routine laboratory use but equipped with a stopcock was employed. It is shown in figure 1. The flattened head has two openings on each side. The tip is rounded sufficiently so as not to puncture tissues, but pointed enough to be thrust through the intercostal muscles after a preliminary incision through the skin and fascia. An intrapleural cavity is created by merely pushing the visceral pleura and lung tissue inward. By practice, the flat nose can be inserted between lobes of the right lung and located in various extracardial regions. By turning the cannula a cavity can be created. The tension of intercostal muscles tends to seal the cannula, but it is advisable to use a purse-string suture or a liberal application of adhesive paste as an added precaution. Connections are made through a leakproof system with an optical segment capsule. The system must be tested repeatedly for leaks. By closing the stopcock at the cannula, calibration of the capsules can be carried out under static conditions by connection with a water manometer. It is important that entry of air during calibrations be meticulously avoided. This happens, for example, if the cannula stopcock is closed while a

negative pressure exists in the recording capsule and tubing and reopened after calibration at atmospheric pressure.

Dogs anesthetized with morphine and sodium barbital were used. Fifteen experiments were performed. Each experiment had two objectives: *1*, to compare pressure variations simultaneously in three of eight different regions of the thoracic cavity, and *2*, to compare effective venous pressures calculated by use of intrathoracic pressure values derived from a number of regions. In these experiments right atrial pressure was inscribed by a Gregg type of manometer. The regions of the chest cavity investigated are diagrammatically indicated in the sketch of figure 2. The registrations are designated as pressure leads. Leads 1,

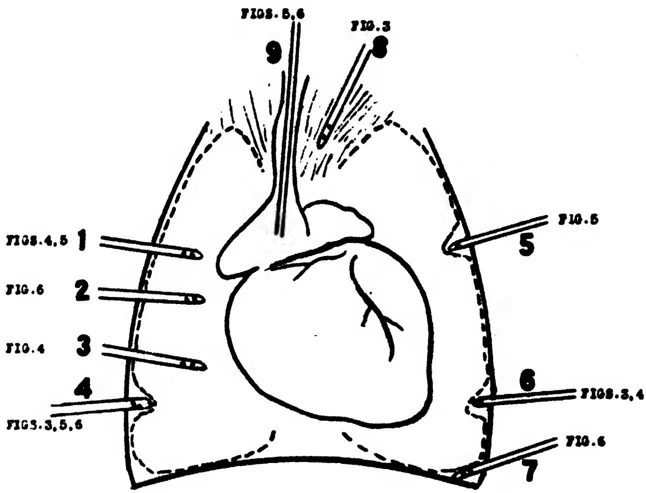

FIG. 2

2, and 3 were recorded from cannulae introduced via fissures between lung lobes so that they lay 2 to 3 cm. to the right of the right atrium, ventricular base, or lower part of the right ventricle, respectively. Lead 4 was taken from the right pleural cavity in the fifth or sixth intercostal space. Leads 5 and 6 were recorded respectively from the left pleural cavity in the region of the second intercostal space and from a region near the apex of the heart. Lead 7 connected with a left supra-diaphragmatic pocket. Lead 8 connected with a pocket made in tissue of the superior mediastinum. A sound was pushed gently from the neck along the large vessels as far into the thorax as possible. This procedure constituted an unsuccessful experimental attempt to discover an upper route to the base of the heart.

RESULTS. Typical pressure changes in the eight different regions shown in figure 2 are illustrated by triple records in figures 3 to 6. Figure 2 also indicates the numbers of subsequent figures in which records by each lead are shown. In comparing pressure changes thus produced around the ventricles with those in

the pleural cavities it is important to develop conceptions as to how respiratory and cardiac phenomena integrate in various regions of the thorax.

Figure 3 shows simultaneous pressure changes around the right atrium (1), right ventricle (3), and left lower pleural cavity near the left ventricular apex (6). A casual inspection of the records reveals that the pressures do not merely fall and rise with inspiration and expiration; on the contrary, they are characterized by definite cardiac oscillations even during expiratory apnea. These can equal values of 20 mm. H_2O. They vary in magnitude and duration at simultaneous moments of systole and diastole in different regions. The duration of atrial systole (AS) and ventricular systole (VS) are approximately indicated on one of the beats. It is apparent that the directional changes vary at simultaneous moments. Thus the pressures tend to decrease initially during ventricular systole around the right atrium (1, also 2 in figure 6), but increase initially around the right ventricle (3). The main trend, however, is for pressure to rise

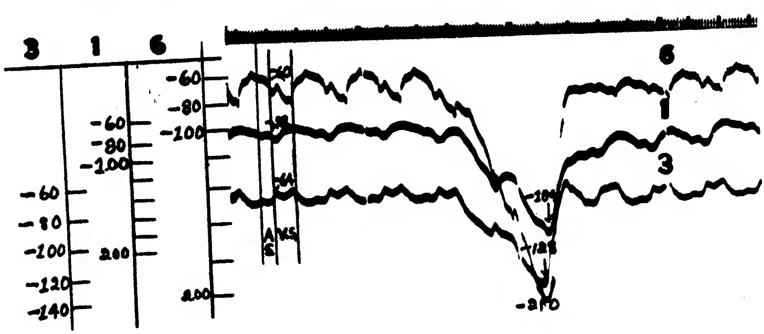

FIG. 3

during systole around the right heart (1, 3) in contrast to areas around the apex (6) in which it decreases. These variations around the heart are obviously due to changes in volume and position of the ventricles as they beat. When transmitted to the lungs and pulmonary air passages they are responsible for the well-known cardiopneumatic effects. The effect of inspiration, also illustrated in this record, cannot be analysed as a simple reduction of pressure; it consists rather of gross deformations of the cardiac variations. Consequently, the maximal decrease in pressure around the heart depends not only partly upon the force of inspiration, but also on the phase of any cardiac oscillation which happens to fall at the end of an inspiration. Such variable pressure changes obviously make it impossible to assign any specific numerical value to pressures around the heart which would be helpful in calculating effective venous pressure

If we take any empirical point, such as indicated in figure 3, the expiratory pressures in leads 6, 1, and 3 are -60, -72, and -64 mm. H_2O respectively. These decrease to -210 -184, and -128, mm. H_2O respectively at the end of a deep inspiration. In other words, inspiration reduces intrathoracic pressure further in the left pleural cavity than around the right atrium and far more than

that around the right ventricle. This is partly due to the fact that the end of inspiration coincides with the more pronounced cardiac drop in lead 6.

In figure 4-A the pressure variations in the left lower pleural cavity (6) already analyzed may be compared with those of the lower right pleural cavity (4) and with those in the mediastinal tissues (8). The cardiac variations present in the latter are probably occasioned by pulsations or tugs of larger vessels. The right lower intrapleural space (4) is apparently devoid of cardiac oscillations; these are apparently not transmitted, either because they are damped out by the spongy lung tissue or because their energy is dissipated more easily through air passages (cardiopneumatic effects). This would seem to indicate that right lower pleural pressures reflect more nearly intrathoracic pressure changes produced by retraction of lungs as modified by skeletal muscle tonus or contraction, thoracic blood content, and lung elasticity. It may be added parenthetically that the

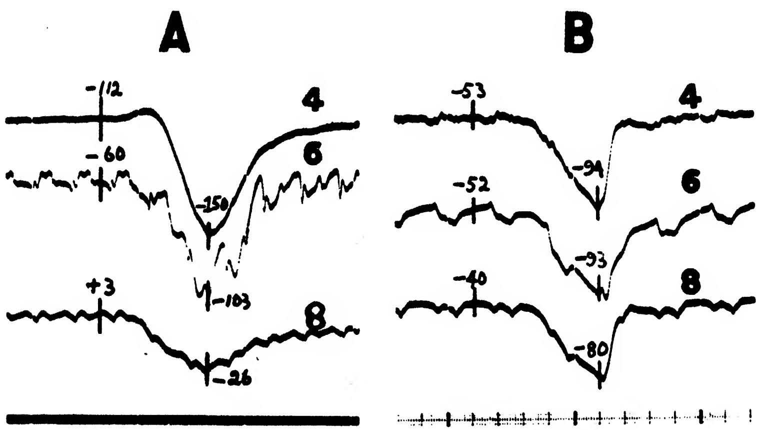

FIG. 4

smooth character of right lower pleural records was another reason why we changed to the use of such pressures in estimating effective venous pressure during our studies on hemorrhagic shock.

Measurement and comparison of pressures in different leads indicate that in this animal left lower pleural pressuse was much less negative than that on the right side. This is exceptional and a surreptitious leak of air may be suspected. However, this difference was approximately maintained during maximum inspiration. The sub-atmospheric pressures in the thoracic cage are apparently not transmitted to the mediastinal tissue during expiration, but a small decrease develops during inspiration. The small positive pressure +3 mm. H_2O is within experimental limits of measurement. It is apparent at once that curves recorded from mediastinal tissue are wholly unsatisfactory for evaluation of intrathoracic or effective venous pressures.

When a partial pneumothorax is produced pressures become equalized in different areas. This is illustrated by segment B from the same experiment after

partial collapse of the lung by injection of air. Not only are the right and left pleural pressures equalized during inspiration and expiration but these changes affect registrations from the mediastinal spaces. Since dogs breathe adequately with such partial collapse of the lungs the study of effective venous pressure in animals with a partial pneumothorax may prove a valuable expedient in types of experiments which do not invoke a state of pronounced hyperpnea.

Figure 5-A shows simultaneous intrathoracic pressure changes from the left upper thoracic cavity (5), around the right atrium (1), the right lower pleural cavity (4), and the right atrium (9). In this experiment inspiratory and expiratory pressures were measured at the very end of diastole for reasons explained later.

The numerals on the record show that right lower pleural pressure (4) is only 4 mm. H_2O higher than extracardiac pressure (1), and that the reduction during inspiration is only 6 mm. H_2O more than around the atrium (1). Experiments such as these indicate good transmission of negative pressure created by muscular

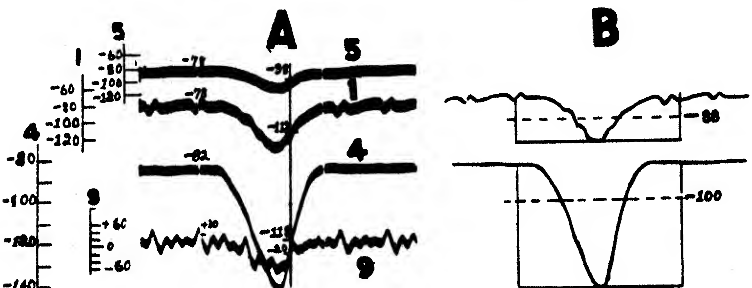

FIG. 5

action to areas around the heart. On the other hand, the upper left thoracic cavity with an identical expiratory pressure (5) is reduced much less during inspiration, presumably because of lesser expansion of the upper thorax.

Figure 5 also serves to illustrate certain difficulties in making pressure comparisons and expedients that may be employed to overcome them. Since atrial pressure (9) also manifests complex pressure variations due to integrating cardiac and respiratory actions, the quandary arises as to how and where these atrial and extracardial pressures should be measured in calculation of effective venous pressure. Several procedures have suggested themselves and have been tried.

1. One may determine the mean atrial pressure for a definite period by integration of curves or estimations based on registered pressures. In such event it is obviously necessary to determine also the mean pressure variation around the heart for the same interval. This has been done in the curves 1 and 4 of figure 5-A which have been retraced in figure 5-B. On these curves the mean values of -88 and -100 mm. H_2O are also indicated. They reveal that the average pressure around the heart for the intervals indicated are about 12 mm. higher than in the lower right thorax. Mean venous pressures were not calculated, but

it is obvious that estimations made from this particular experiment would have to take these mean pressure differences into account if absolute values for effective venous pressures and not merely trends are wanted. It remains questionable, however, whether such calculations of effective venous pressure really represent the true filling pressures. At any event, estimations made in this way seem to have too little value in cardiodynamics to warrant the trouble involved.

2. We have previously pointed out (2) that mean atrial pressure measured during diastole of a selected beat is probably of greater significance as far as

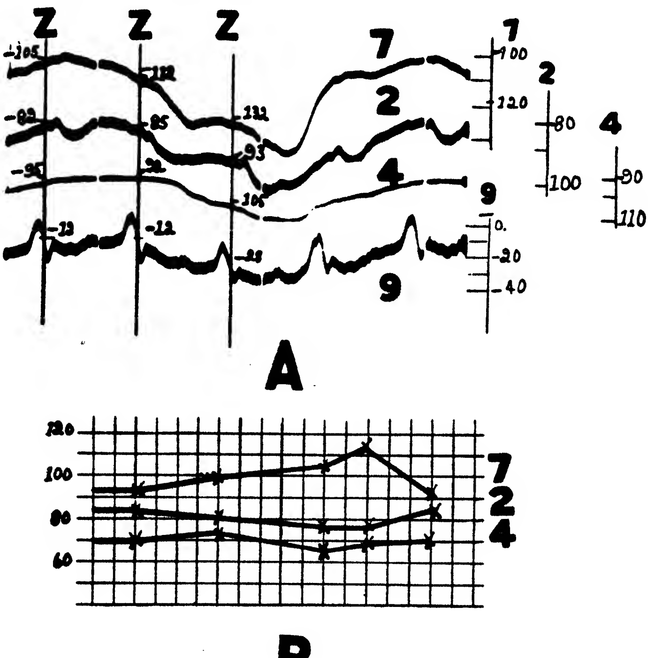

FIG. 6

ventricular filling is concerned. The pressure which exists in the atrium during ventricular systole is obviously not concerned in filling. Consequently, it would be a better procedure to determine the mean pressure during diastole of a single heart cycle both within the atrium and in an intrathoracic pressure curve.

3. Since the magnitude of ventricular filling, i.e., ultimate diastolic size, is actually determined by the pressure difference at the end of diastole—i.e., after termination of atrial systole—the instantaneous pressure which exists at this moment probably constitutes a reasonable and certainly the simplest criterion of the venous pressure component. Since this point was labeled Z on atrial pressure curves in an earlier communication (2) we shall refer to it as the Z pressure. The

algebraic difference between this instantaneous atrial pressure and that which which exists simultaneously around the heart determines the effective venous pressure for that beat. The pressure measurements indicated in figure 5-A were actually made in this way, but the procedure is illustrated better by reference to figure 6-A. In this figure simultaneous records are shown which were derived from the left supra-diaphragmatic region (7), from a region around the right ventricular base (2), from the lower right pleural cavity (4), and from the right atrium (9). The line Z is drawn through all the curves at the end of atrial systole and demarcates simultaneous Z pressures. The curves show a reduction in all Z pressures during inspiration, the values being inscribed directly on the curves. It will be noted that the effect of an inspiration consists in reducing the Z pressure of the first two beats in lead 2 by 3 and 11 mm. respectively, whereas the Z pressure of corresponding beats in lead 4 is reduced by 3 and 10 mm. respectively. The reduction in left sub-diaphragmatic pressures (7) on the other hand is much greater. This illustrates again the accuracy with which changes of intrapleural pressure produced by muscular action are transmitted from pleural surfaces to the interior of the chest.

The effective venous pressure calculated for successive beats in relation to values derived from lead 7, 2, and 6 respectively are plotted in figure 6-B. They show as good correspondence as can be expected from calculations based on use of leads 2 and 4, but even show an opposite trend when values from lead 7 are used.

The analysis of many records such as these has revealed that instantaneous Z pressures taken at the end of an expiration or during a short expiratory pause agree within 5 mm. H_2O in various regions around the right ventricle (leads 1, 2, 3), and in 13 experiments averaged only 9.3 ± 1.4mm. H_2O higher than that in the right lower pleural cavity.

Discussion. Our analysis of pressure relations from eight different thoracic regions, only samples of which can be presented, led to definite conclusions with regard to their employment in calculations of effective venous pressure.

Owing to the intricate integration of cardiac and respiratory pressure variations which occur in certain regions of the chest, special expedients are required in order that pressures in different regions may be compared. We have tried to do this by using average pressures over a period of time, mean diastolic pressures of a selected beat during expiration, and the Z pressure, i.e., the instantaneous pressure at the very end of diastole. The latter has proved most informative and is at the same time a simple measurement.

By use of all these criteria we have confirmed the conclusions of Brookhart and Boyd that pressures vary sufficiently in different regions of the thorax so that indiscriminate use of pleural pressures can lead to gross differences in calculations of effective venous pressure and even to opposite trends. Careful studies have shown, however, that the pressures in the right lower pleural cavity correspond closely enough to pressures around the right heart to be serviceable for calculation of effective venous pressures, provided proper measuring technique is used.

In fact it appears probable that right pleural pressure represents the funda-

mental effects produced by changes in tonus and contraction of right-sided respiratory muscles undistorted by phasic pressure changes contributed by the heat of the heart. We have pointed out in discussions of figures 5 and 6 that the effects of inspirations are faithfully transmitted to the pericardial surfaces and that deviations in the extracardiac pressure are probably due to superposition of cardiac variations. Since the latter represent dissipated cardiac energy, it remains questionable whether uncomplicated intrapleural or complicated extracardiac pressures represent the intrathoracic component more reliably in estimations of effective venous pressures. At any event, any theoretical advantage of using extracardiac pressures for determining the intrathoracic component of effective venous pressures is offset by the practical drawbacks, *a*, that the pressure varies somewhat in different regions of the ventricle, and *b*, that it is more difficult to maintain a free opening of cannulae in the interior of the chest when experiments last for a number of hours.

Our results confirm the observations of Brookhart and Boyd that pressures around the heart are generally less sub-atmospheric than in many pleural regions. As accurately as determinable, the difference is of the order of 10 mm. H_2O as far as the right lower pleural cavity is concerned. It is our opinion that the somewhat higher extra-cardiac pressure is not occasioned as Brookhart and Boyd postulate. They attribute it to the fact that the heart and mediastinal structures prevent the lungs from assuming a freely inflated state in the interior of the chest, thus reducing the effective retractive force exerted on pericardial structures. Were this the case, the increased negative pressure created in the right lower pleural cavity during inspiration would hardly be transmitted to regions around the heart as faithfully as indicated in figures 5 and 6. Their arguments and experimental evidence would seem to apply to conditions in which additional or unnatural obstruction exists, not to the anatomical structures already in the chest. It is well known that during growth the lungs have shaped themselves so as to conform to the solid structures in the middle of the chest. It is conceivable that pathological dilatation of the heart which develops after the lungs have been shaped to the normal structures might operate to obstruct inflation of the internal surfaces of the lungs. It is altogether improbable, however, that this plays an important role normally, or under experimental conditions with which we have been concerned. It is our opinion that the superimposed cardiac variations are responsible for elevating pressures produced by pull of the lungs around the heart to a certain degree.

Conclusions. On the basis of our studies we conclude that, although effective venous pressures cannot be calculated with finite accuracy, computations based on employment of right lower pleural pressures are fully as reliable as most of our biological data. We favor continued use of right lower intrapleural pressures because *1*, it remains debatable whether extracardiac pressures represent the truer component, *2*, the registration of the latter offers greater chance of error in prolonged experiments and *3*, properly calculated changes of right intrapleural pressure vary directionally and fairly proportionately with pressures around the heart.

SUMMARY

1. Technical errors introduced by various procedures employed to measure intrathoracic pressures, such as creation of air pockets or introduction of balloons, are discussed.

2. Intrathoracic pressures were recorded by optical capsules from 8 separate regions of the heart by a method which requires introduction of very minimal quantities of air into mechanically created pockets.

3. Records of intrapleural pressure from the upper regions of the left side and lower regions of the right side of the thorax consist of smooth curves of subatmospheric pressure which decrease further during inspiration. Changes in these pressures are occasioned by modifications in tonus and contraction of respiratory muscles, blood content of the chest, and elasticity of lung tissue.

4. Records from artificial pockets around the right heart and in the left lower pleural spaces adjacent to the apex of the heart show superposition of conspicuous cardiac variations on respiratory variations. It is suggested that these superadded variations rather than distortion of lung tissue by the heart and mediastinum are responsible for the existence of somewhat higher pressures around the heart than in the pleural cavities.

5. In order to compare variable pressures around the heart with constant pressures found in some pleural leads special methods for comparison were devised. Of several methods tried, comparison of instantaneous pressures at the end of a selected diastole, or so-called Z pressure, is an adequate and simple procedure which is easily related to atrial pressures as well.

6. Comparison of such Z pressures in various intrathoracic regions reveals that pressures recorded from the right lower thoracic cavity are only about 10 mm. H_2O higher than those derived around the right heart and change directionally with them. Since it remains a moot question as to which of these represent the truer intrathoracic component, such records may certainly be used in calculating trends of effective venous pressure. The error involved is no greater than exists in most of our biological data. Intrathoracic pressures recorded from the upper regions of the chest, from the whole left side or regions above the diaphragm and from the mediastinum, are unreliable for one reason or another.

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STUDIES OF THE VENOUS DRAINAGE OF THE HEART[1]

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Received for publication August 20, 1947

Anatomically, the anterior cardiac veins, the coronary sinus and its tributaries, all emptying directly into the right atrium, and the Thebesian and other vessels communicating with the heart cavities, constitute the major potential venous drainage pathways of the heart. The extent to which each contributes to the venous drainage of the heart has been the subject of many investigations. Numerous physiological studies have formed the basis for the belief that blood from the right coronary artery is ultimately drained almost entirely by way of the Thebesian vessels emptying into the right heart (1–3), while blood from the left coronary artery is drained largely through the superficial veins of the left heart into the coronary sinus (4–6). The results of the same studies have also led to the presumption that the extracoronary sinus drainage occurs almost entirely by way of the Thebesian channels into the heart cavities. The evidence for these conclusions has been based largely on experiments with heart-lung and isolated heart preparations, in which the quantitative and cyclic changes in pressure gradients between different parts of the coronary vascular system and the heart chambers could be significantly altered as the result of the procedures and methods employed. In none of these experiments was the venous drainage from the anterior cardiac veins emptying directly into the right atrium measured separately or adequately separated from the so-called "Thebesian drainage." With the present knowledge of the important role played by the anterior cardiac veins (7), certain revisions are necessary regarding the functional pathways for coronary outflow. For example, in the anesthetized, open-chest dog, blood from the right coronary artery drains for the most part, and in some instances almost entirely, by way of the anterior cardiac veins to the right atrium, and not primarily through Thebesians as previously held. Because of this fact, and in view of the extensive anastomoses between the anterior cardiac veins and those superficial veins which ultimately empty into the coronary sinus (7), it is obvious that coronary sinus occlusion alone will not necessarily force coronary venous blood to drain exclusively by way of Thebesian channels. Hence it is necessary to re-evaluate several concepts regarding the coronary venous circulation in the light of more recent experimental studies. Of the studies presented

[1] The expenses of this investigation were defrayed by a grant from the Commonwealth Foundation.

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here, a part is complete, and a portion is of an exploratory nature to point the way to future work.

Studies and Procedures. The studies include *a*) determination of venous drainage into the coronary sinus and anterior cardiac (AC) veins with consideration of source, volume and constancy of the relationship to coronary inflow under a variety of cardiodynamic states, and *b*) the effects on the heart of acute and chronic occlusion of the major superficial cardiac veins of the right and left heart, either separately or together, as revealed by injection of the hearts post-mortem, measurements of coronary inflow and utilization of alternate pathways of venous drainage. For this, using anesthetized, open-chest dogs, blood flow determinations were made in different coronary arteries and veins by means of visual (8) and optical recording (9) rotameters, and by the orifice meter (10). Intravascular and intraventricular pressures were recorded optically by Gregg pressure manometers (11). The application of these instruments, the preparation of the animal, and vessel cannulation have been described previously (12). Further details are contained in the text.

RESULTS. *Venous drainage into the coronary sinus.* From the anatomical distribution of coronary veins over the left ventricular surface it would be expected that the coronary sinus into which they drain would receive the major portion of the blood originally supplied by the left coronary artery. The situation in the left heart would then be analagous to that in the right heart, in which the prominent anterior cardiac veins were suspected of being, and found to be, the major drainage system for blood supplied by the right coronary artery. To measure coronary sinus flow, a cannula was inserted into the right atrium, passed into the sinus and tied in place. A second cannula was tied in the right atrium near the inferior cava. A rotameter was then interposed between the two cannulae to complete the sinus-to-atrium circuit. The left coronary artery inflow, and/or the right, was measured simultaneously by a second rotameter, the cannula for which was tied into the coronary orifice as previously described (12).

With this technic, it was found that almost all the coronary sinus flow arose from the left coronary artery. Temporary clamping of the left coronary artery at its ostium immediately reduced coronary sinus flow to values approximating 2 to 3 cc/min., or 5.0 to 7.5% of the original flow values (12 determinations in 4 experiments; see table 1, exp. 1, 20 *a*). In 3 additional experiments, temporary occlusion of the right coronary artery alone reduced coronary sinus flow by a detectable amount in only 2 of 11 determinations, even when right ventricular pressure was considerably elevated by mechanical constriction of the pulmonary artery[4] (table 1, exp. 3, 8, 21). However, when temporary occlusion of the right coronary is superimposed on an existing left coronary occlusion (3 experiments, 5 determinations), the coronary sinus flow dropped slightly more by falling further from 2.0, 2.5, 2.5, 3.0 and 3.0 to 1.0, 1.0, 1.0, 1.0 and 1.5 cc., thus indicating that possibly 1.0 to 2.0 cc. of blood was entering the coronary sinus from

[4] In 4 experiments (4 determinations), temporary occlusion of the coronary sinus also failed to cause a reduction in right coronary inflow.

the right coronary artery under the favorable conditions imposed by left coronary occlusion (table 1, exp. 2). This small drainage of the right coronary artery into

TABLE 1

EXP.	MEAN AORTIC PRESS.		ARTERIAL INFLOW		VENOUS OUTFLOW		RATIO INFLOW/OUTFLOW		REMARKS
	Before	During	Before	During	Before	During	Before	During	
	mm.Hg	mm.Hg	cc./min.	cc./min.	cc./min.	cc./min.	%	%	
			Left cor.		Cor. sinus				
1	85	85			55.0	2.0			Effect of clamping left coronary artery on coronary sinus flow
2	85	85			55.0	1.0			Effect of clamping right and left coronary arteries on coronary sinus flow
3	78	78	45	47	35	37	78	79	Effect of clamping right coronary artery on cor. sinus flow following pulmonary art. constriction
4	70	67	49	60	36	42	73	70	Effect of pulmonary artery constriction (4 min.) with partial aortic blood pressure compensation*
5	90	52	55	54	40	42	73	78	Effect of slow spontaneous fall in aortic blood pressure (20 min.)
6	55	55	62	85	43	60	69	71	Injection of 0.1 cc. theamin into left coronary artery
7	70	70	67	55	45	0	67		Effect of clamping coronary sinus on left coronary artery inflow
			Rt. & left cor.		Cor. sinus				
8	60	60	71	60	46	46	65	77	Effect of clamping right coronary on coronary sinus flow
9	70	71	60	86	40	58	66	67	Effect of pulmonary artery constriction (7 min.) with aortic blood pressure compensation*
			Rt. cor.		AC veins				
10	70	70	7	19	2.6	13.0			Pulmonary artery constriction vs. AC vein flow. Flow measured in 1 of 3 major AC veins. Approx. 50% of AC flow from right coronary artery

the coronary sinus is somewhat less than that reported by others using the heart-lung preparation (13, 25). The residual and just detectable coronary sinus flow remaining after essentially complete acute coronary artery occlusion presumably

TABLE 1—*Continued*

EXP.	MEAN AORTIC PRESS.		ARTERIAL INFLOW		VENOUS OUTFLOW		RATIO INFLOW/OUTFLOW		REMARKS
	Before	During	Before	During	Before	During	Before	During	
	mm.Hg	*mm.Hg*	*cc./min.*	*cc./min.*	*cc./min.*	*cc./min.*	%	%	
11	90	92	8	11.5	5.0	11.0			Pulmonary artery constriction. Flow measured in 1 of 3 major AC veins. Approximately 70% of AC flow from right coronary artery
12	95	92			4.2	13.2			Coronary sinus occlusion (4 min.) on AC vein flow. Flow measured in AC vein nearest pulmonary conus
13	90	90			5.6	12.6			Coronary sinus occlusion (2 min.) on AC vein flow. Flow measured in 3 of 4 major AC veins
14	67	67*	16	19					Pulmonary artery constriction vs. rt. cor. flow with all major AC veins previously ligated. Rt. ventricular pressure rose from 15/0 to 30/2 mm. Hg
15	72	72	9	12.5					Pulmonary artery constriction vs. rt. cor. flow with all grossly visible AC veins and cor. sinus previously ligated (5 min.). Rt. ventricular pressure rose from 13/0 to 42/2 mm. Hg
			Left circ.						
16	103	102	20	21					Coronary sinus occlusion (6 min.) vs. left circ. flow (minimal effect)
17	80	80	20	16					Coronary sinus occlusion (3 min.) vs. left circ. flow (maximal effect)
			Right cor.						
18	92	90	14	6					Occlusion of major AC veins vs. right coronary flow (maximal effect)
19	100	95	11.5	11.5					Occlusion of major AC veins vs. right coronary flow (minimal effect)
			Right cor.		Cor. sinus				
20*a*	105	105	13.0	15.0	63.0	3.0			Effect of clamping common left coronary artery on coronary sinus flow
20*b*	105	105	15.0	0	3.0	1.5			Right coronary clamped after previous clamping of left coronary
21	68	68	14.5	0	34.0	30.0			Effect of clamping right coronary artery on coronary sinus flow (maximal effect)

*Aortic blood pressure compensation was accomplished by manual adjustment of a clamp placed on the lower portion of the thoracic aorta.

arose from small twigs of the coronary arteries, which were always found (by postmortem injection and inspection) to lie central to the sites of coronary artery occlusion.

Although essentially all the blood in the coronary sinus appears to arise from the left coronary artery, not all the left coronary inflow leaves by this channel. Measurements in 3 different experiments revealed that 64 to 83% of the blood from the left coronary artery drained through the coronary sinus (determined during temporary occlusion of the right coronary artery). These results are in general agreement with previous observations by others using the heart-lung and isolated heart preparation (13).

Early investigators, using heart-lung preparations (4–6), observed that coronary sinus outflow indicated semi-quantitatively the changes but not the magnitude of left coronary or total coronary inflow. More recent investigators (2, 15–17) have challenged the general application of this relationship on the basis of experiments performed with the heart-lung preparation, isolated heart, fibrillating heart and the perfused dead heart. From a practical standpoint the issue would seem to be of little import, since simple and adequate methods are now available for the direct measurement of coronary inflow. However, in view of the quite unnatural preparations which were generally used, the abnormal pressure relations created in many of the experiments, and because in some instances the deductions were made on the basis of indirect evidence, it was of interest to repeat certain experiments under conditions in which the normal physiological state of the heart was disturbed as little as possible.

For the following, only 2 animal experiments were performed, representing a total of 60 observations. With rotameters to measure coronary inflow and coronary sinus outflow, the effects of various procedures were determined. Changing aortic blood pressure, elevation of right ventricular pressure, saline and blood infusion and the intra-arterial injection of drugs did not greatly alter the relationship of coronary sinus outflow to left coronary inflow, and in no instance would a significant change in left (or total) coronary inflow have been incorrectly indicated by the corresponding coronary sinus outflow. In one experiment the coronary sinus flow constituted 79% of left coronary inflow ($\pm$ 3.8); in the other experiment this value was 73% ($\pm$ 2.6). Also, in the latter experiment coronary sinus flow equaled 64 to 68% of total coronary inflow (6 determinations). (See fig. 1 and table 1, exp. 4–6, 9).

The observations on this relationship during constriction of the pulmonary artery are of particular interest. Johnson and Wiggers (15), Katz, Jochim and Bohning (14), Moe and Visscher (2), and Visscher (17) have stressed the importance of right ventricular pressure in modifying coronary flow. Primary emphasis is placed upon the reasoning that elevation of right ventricular pressure, by increasing the resistance against which the Thebesian vessels may discharge venous blood into the right ventricular cavity, causes a diminished blood supply to the right heart. The observed concurrent increase in coronary sinus outflow with elevation of right ventricular pressure is interpreted to indicate that blood is shunted away from the Thebesians of the right heart, or that there is an

actual retrograde flow of Thebesian blood from the right ventricle to veins emptying into the coronary sinus. While such deductions may conceivably apply under hemodynamic conditions artificially induced in certain types of preparation, they do not constitute a logical explanation for the findings obtained under more nearly normal physiological conditions. As already indicated, recent

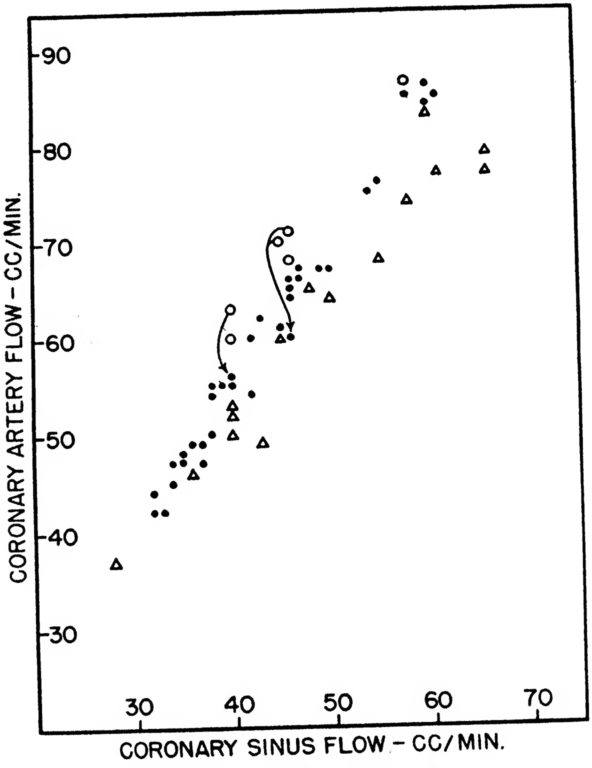

FIG. 1. Graph showing relationship of common left coronary inflow or total coronary inflow (ordinate), and coronary sinus outflow (abscissa), under different dynamic conditions. Dots and triangles, common left coronary inflow in 2 experiments. Circles, total coronary inflow (right and left). Dots at end of arrows, common left coronary inflow after clamping right coronary artery.

studies with the heart beating *in situ* have demonstrated that most of the venous drainage of the right heart is through the anterior cardiac veins rather than through Thebesian channels to the right ventricle (7). In the present study, elevation of right ventricular pressure increased both right and left coronary inflows considerably (24 experiments), which fact alone is sufficient to account for the increased coronary sinus outflow (table 1, exp. 4, 9–11). In addition, the flow through the anterior cardiac veins increased greatly (table 1, exp. 10, 11).

These results indicate that in any one experiment, with reasonably normal

hemodynamic conditions prevailing, changes in coronary sinus flow may serve as a directional indicator of left coronary inflow (and presumably also of total coronary inflow), although the actual volume of coronary artery flow cannot be accurately predicted since the relationship of coronary sinus flow to coronary artery flow may vary in different experiments.

The experiments of Katz, *et al.*, (14) demonstrating that the coronary sinus outflow may exceed total coronary inflow, would seem to have little physiological significance in that they indicate what can be made to happen in the heart by creating highly abnormal and unphysiological pressure gradients. As already indicated in experiments with hearts beating *in situ*, a temporary occlusion of both coronary arteries decreases coronary sinus outflow to almost zero within 10 to 20 seconds (observations made before beginning of fall in blood pressure, table 1, exp. 2, 20 *b*). If significant retrograde flow through the Thebesian vessels were possible within physiological limits, the pressure gradients between the ventricular cavities and the coronary vessels should be optimal for its demonstration during the period of complete occlusion of the coronary arteries. This confirms the findings of Stella (18).

Venous drainage into the anterior cardiac veins. The functioning of these venous channels in the open-chest, anesthetized dog has been partially considered previously (7). The flow through the AC veins was found to arise almost, if not entirely, from the coronary arteries, for when both coronary arteries were occluded close to their respective ostia, the anterior cardiac vein-flow dropped to essentially zero, or at most, 1.0 cc. per minute. Examination of the hearts at the end of experiments in which residual flow persisted showed twigs of the right and/or left coronary artery which had not been occluded by the experimental procedure. Thus in these veins, as in the case of the coronary sinus, there would appear to be essentially no extracoronary source of blood supply. However, unlike the coronary sinus, the AC vein flow arises from both coronary arteries rather than almost entirely from one coronary artery. When all major AC veins were cannulated and their collective flow measured, most of it was found to arise from the right coronary artery (determined by temporary clamping of the right coronary artery). The remainder of the AC vein flow (varying from 0 to 16 cc/min. in 12 different experiments and occasionally constituting most of the AC vein flow) arose from the left coronary artery (determined by temporary clamping of the latter artery). This amount is sufficient in most instances to account for much of the left coronary inflow which does not appear in the coronary sinus, but whether there is a quantitative transfer has not been established experimentally because of the technical difficulty in measuring flow simultaneously in both coronary arteries and in all the superficial venous channels of the heart.

No consistent relationship between AC vein flow and right coronary flow could be demonstrated. In 7 different experiments (80 determinations) in which all the major AC veins were cannulated, the ratios of AC vein outflow to right coronary inflow under different conditions varied from 0.6 to 0.8, 0.81 to 1.43, 0.3 to 1.0, 0.5 to 0.75, 0.73 to 1.37 and 0.57 to 0.9. Considerable variations

in the ratio would be expected since the AC veins drain certain areas of the heart which are supplied by both right and left coronary arteries. Hence a small change in the amount of blood contributed by the left coronary artery can have a relatively large effect on the ratio of total AC vein flow to right coronary artery flow.

The effects of acute and chronic occlusion of the major superficial cardiac veins. Although the importance of the superficial (non-Thebesian) venous pathways in the normal drainage of the right and left heart should be emphasized, investigators have demonstrated that the major collecting vein, the coronary sinus, may be experimentally occluded in acute or chronic preparations without apparently causing serious interference with the functioning of the heart. Anrep, Blalock and Hammouda (13) have observed that when the coronary sinus was clamped in heart-lung preparations, total coronary inflow decreased 25%, and concluded that the remaining 75%, which had presumably drained via the coronary sinus, had now become diverted into the Thebesian drainage channels. Robertson (19) and Beck and Mako (20) have reported that the coronary sinus could be occluded in chronic experiments with a mortality of 0 to 2%, in spite of the fact that these hearts examined postmortem showed subepicardial scarring of the left ventricle. Robertson (19) states that in this situation, "Thebesian vessels dilate so readily that the venous blood of the left heart is rapidly forced into the left ventricular cavity and no congestion occurs," (in most hearts).

The belief that Thebesian vessels become the alternate pathway for the coronary sinus blood flow lacks direct experimental substantiation. The fact that the anterior cardiac veins have been shown to drain a considerable portion of right and left coronary inflow and to have extensive anastomotic (collateral) connections with the tributaries of the coronary sinus system suggested that the anterior cardiac veins were in a favorable position to carry off blood diverted from the coronary sinus under these conditions.

A similar situation may exist with respect to drainage of the right coronary artery. Although acute occlusion of the major AC veins reduces right coronary inflow by 0 to 63%, it is not possible to determine how much of the residual inflow represents blood normally drained by way of the coronary sinus and how much represents flow which has been shunted from the AC veins to the coronary sinus under the artificial conditions imposed by the procedure. That a portion of right coronary inflow may normally drain through the coronary sinus is suggested by the findings exemplified by table 1, exp. 20 *b*, 21.

A. *Acute occlusion.* Our findings obtained in acute experiments show that coronary sinus closure causes congestion of the left heart, a greatly elevated venous pressure in the coronary sinus and great cardiac vein (21, 22), but only a relatively small reduction in flow in the left coronary or its major branches, averaging 8% in 10 dogs (table 1, exp. 7, 16, 17). However, the venous outflow measured simultaneously in several major anterior cardiac veins increased greatly (table 1, exp. 12, 13). The maximum extent to which the blood normally draining into the coronary sinus may be diverted to anterior cardiac venous channels was not determined because, owing to technical difficulties of the procedure,

cannulation and measurement of blood flow from only the large anterior cardiac veins was attempted. However, the experiments strongly suggest that the anterior cardiac veins may serve as a very important, if not the primary, alternate route during occlusion of the coronary sinus.

Similar responses occur when the major venous drainage channels of the right heart, the anterior cardiac veins, are ligated in acute experiments. Anterior cardiac venous pressure arises, the right ventricle becomes congested and right coronary inflow may decrease from 0 to 63%, averaging 21% in 8 different experiments (table 1, exp. 18, 19). Even under these conditions, in which most of the normal drainage channels for the right coronary artery (as determined by occlusion of the right coronary artery, 7) have been removed, increasing the load upon and pressure within the right ventricle (pulmonary artery constriction) increases right coronary artery inflow (table 1, exp. 15).

In acute experiments, hearts have been found to survive for several hours following occlusion of both the coronary sinus and all grossly visible anterior cardiac veins.[5] The fact that under these conditions, increasing the load on the right ventricle still resulted in a considerable elevation in right coronary inflow, indicates that these hearts still had at their disposal reasonably efficient venous drainage channels (table 1, exp. 15). The potential venous drainage channels remaining were the small anterior cardiac veins, any unoccluded veins emptying into the right atrium adjacent to the coronary sinus, collateral communications with extracardiac veins and Thebesian vessels.

In subsequent experiments, an effort was made to deprive the heart of all venous drainage pathways exclusive of Thebesian vessels, so that the ultimate rôle of the latter could be more positively identified. Three acute experiments were performed in which all grossly visible or otherwise accessible anterior cardiac veins, the coronary sinus and extracardiac veins found in the vicinity of the base of the heart were occluded by ligature. In all instances both ventricles became dilated, very firm and markedly hemorrhagic with many dark petechiae and ecchymotic areas. The atria appeared normal in color and were without hemorrhagic areas. The superficial veins over both ventricles became distended and tortuous. All of the hearts continued to beat for at least 2 hours, but with a gradual fall in blood pressure. Thirty to forty minutes after occlusion of the veins there invariably appeared several previously unnoticed extracardiac veins running laterally or superiorly from the fatty tissue surrounding the cardiac base. The hearts continued to beat and appeared in no worse condition after these veins were also ligated.

Postmortem examination of the hearts uniformly revealed extensive hemorrhage throughout the entire thickness of the right ventricular wall. The left ventricular wall was similarly affected except that its inner third and the ventricular septum appeared relatively normal. None of the hearts showed any apparent abnormality of the auricular-ventricular septum. Close examination of the area surrounding the opening of the coronary sinus revealed that in all of

[5] Following the initial occlusions, additional AC veins became visible which were also occluded.

the hearts there were one or two small veins with separate openings into the right atrium which had not been included in the ligatures placed about the termination of the coronary sinus. In addition to the one or more veins known to have been unoccluded, there were undoubtedly other small veins emptying into the right auricle which were not noticed or could not be positively identified on gross inspection. In two of the experiments the coronary venous system was injected immediately postmortem with a gelatin-dye mixture through a cannula tied into the coronary sinus. The entire system of superficial veins was readily injected, and the dye was found to appear in many small veins about the roots of the great vessels and also in several of the adjacent intercostal veins. No dye was found in the non-hemorrhagic, inner layers of the left ventricular wall or in the basal portion of the interventricular septum.

Considering the very high venous pressures (approaching or exceeding aortic pressure, 21, 22) created by the occlusion procedures and the extensive anastomoses among the coronary veins, it is not improbable that a few small, unoccluded veins would be adequate under such conditions for the drainage of the total coronary blood flow, the volume of which must have been greatly reduced judging from the appearance of the hearts. These attempts to deprive the heart of all non-Thebesian venous channels were therefore considered to be unsuccessful, and the ability of the hearts to survive for 2 hours or more could not necessarily be attributed to the drainage of blood by Thebesian vessels.

However, the normal appearance postmortem of the atria, the inner left ventricular wall and basal septum, and the failure of the gelatin-dye mixture to enter these myocardial areas when the superficial veins were injected suggests that these portions of the heart may have a separate venous drainage system, possibly involving Thebesian channels. If this is the case, communications between the Thebesian system and the superficial veins of the heart would not appear to be very extensive, since dye was not found to enter these areas. It is, therefore, unlikely that the drainage system for the septum and inner left ventricular wall (not identified in these experiments) could have served as an important alternate route for the escape of coronary venous blood from the hearts subjected to acute superficial vein occlusion.

B. *Chronic occlusion.* The effects of ligating the coronary sinus and all accessible anterior cardiac veins in chronic experiments have also been investigated. For each experiment, a two-stage operation was performed using morphine and ether anesthesia. Careful dissection and ligation of all but the extremely small veins at their points of entry into the right atrium caused immediate congestion of the right ventricle but not of the left ventricle. At the time of the second operation (1–2 months later) the right ventricle was no longer congested, but following ligation of the coronary sinus both ventricles became congested. Similar changes in the appearance of the corresponding ventricles occurred when the anterior cardiac veins and coronary sinus were ligated in the reverse order. Two months after the final ligation, the surviving dogs were anesthetized and hearts exposed. Although the congestion of the myocardium had disappeared, rather extensive areas of the right ventricle exhibited superficial scarring. (Subsequent microscopic examination revealed cellular infiltration

and a moderate increase in connective tissue with a proportional decrease in muscular elements.) Gross examination of the hearts *in situ* revealed several sizeable anastomoses between the superficial veins of the heart and extracardiac veins. In figure 2 are drawings of one of the hearts which had been injected postmortem with a gelatin-dye mixture through one of the extracardiac veins. The entire superficial venous system became well filled with dye as did the extracardiac venous connections emerging from the heart in several regions. In

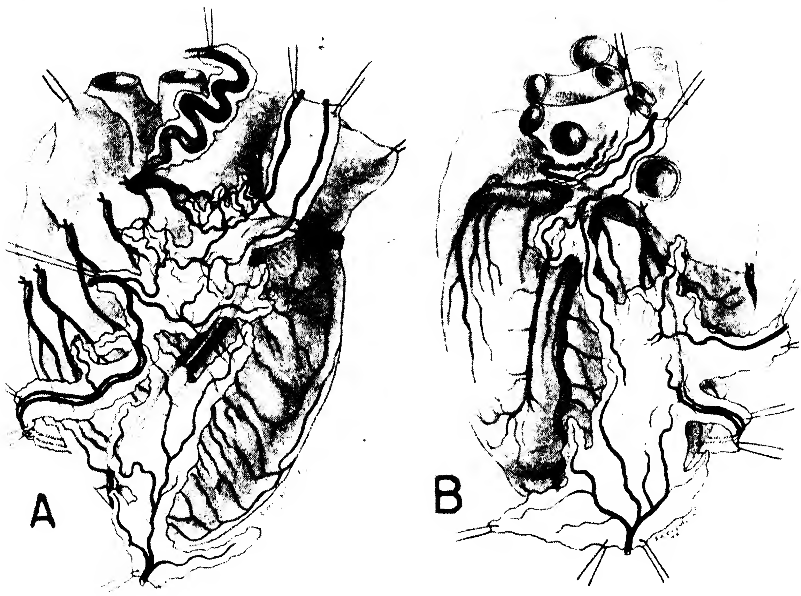

FIG. 2. Drawings of the anterior (A) and posterior (B) views of a dog heart following chronic ligation of the anterior cardiac veins (4 months) and coronary sinus (2 months), showing the extracardiac superficial venous channels. Heart injected *in situ*, postmortem, through an extracardiac vein with a gelatin Evans Blue mixture. Note injection of the superficial veins of both ventricles and of the extracardiac veins.

addition, certain anterior cardiac veins, which apparently were not large enough to have been noticed at the time of previous operations, had enlarged to become quite prominent. Examination of the inner surface of the heart failed to demonstrate plugs of injection material or enlarged Thebesian openings.

Thus the development and enlargement of extracardiac venous anastomoses is quite similar to that observed on the arterial side of the coronary circuit following occlusion of one or both coronary arteries (23, 24). The fact that extracardiac anastomoses develop following progressive occlusion of the coronary arteries or veins would seem to indicate that the Thebesian vessels are not, in themselves, sufficient to function as adequate channels for either supplying blood to, or draining blood from, the myocardium. It is even less likely that they would function

to any great extent in either capacity when the normal channels for arterial and venous flow are left intact.

The observations presented here, together with previously reported findings, form the basis for a re-evaluation of certain concepts of the coronary circulation as well as the associated methods of experimental approach.

It is not to be claimed that these studies with the heart beating *in situ* in the anesthetized dog necessarily indicate the nature of occurrences in the normal, unanesthetized animal. However, the experimental findings thus obtained may be coordinated into a reasonably consistent interpretation of physiological events which could not be accorded many previous studies made under more artificially controlled or quite abnormal conditions. The following conclusions are, nevertheless, tentative and are made with the distinct reservation that they apply only to the heart of the anesthetized, open-chest dog. Their "extrapolation" to the unanesthetized animal or to humans does not seem advisable without further investigation.

SUMMARY AND CONCLUSIONS

A correlation of the anatomical and physiological findings presented here and in previous work gives a simpler view than heretofore presented of certain aspects of the coronary circulation. The experimental findings and conclusions based on 50 dog experiments are:

In the anesthetized, open-chest dog with the heart beating *in situ*, blood-flow measurements in the coronary arteries and superficial veins indicate that the right coronary artery is the major source of blood supply and the anterior cardiac veins the major drainage system of the right ventricle. Similar measurements show that the left coronary artery and the coronary sinus with its contributing veins constitute the corresponding arterial and venous systems of the left ventricle. Thus the superficial veins of the heart appear to play the dominant role in draining the coronary vascular bed.

The veins (and arteries) of each heart overlap anatomically in the adjacent portions of the ventricles, and each system has many extracardiac anastomoses. The anastomoses between the anterior cardiac veins and those contributing to the coronary sinus are extensive. When either superficial coronary venous system is acutely blocked, the coronary inflow to the respective myocardium is usually only moderately reduced, and the venous blood presumably passes into the right auricle by way of collateral communications to other unblocked venous channels.

With as complete as possible ligation of the entire superficial cardiac venous system in acute experiments, the hearts generally survive for several hours and respond to increased load with augmented coronary inflow. In chronic occlusion experiments, injection of the venous system of such hearts shows the presence and extensive development of large and numerous extracardiac venous anastomoses and several enlarged, unoccluded anterior cardiac veins. The conclusion is inescapable that such hearts still had at their disposal an extensive system of superficial cardiac venous drainage channels. It is a matter of conjecture what rôle, if any, the Thebesian vessels may have played in the venous drainage of

the heart during the time the collaterals and anastomoses were enlarging. However, the prompt and extensive development of a substitute system for draining the superficial veins of the heart offers little support to the belief that the Thebesian vessels are adequate or serve in a major capacity as an alternate system for venous drainage.

The experiments emphasize the functioning of the superficial cardiac veins and tend to minimize any rôle which the Thebesians may play in coronary drainage. While it cannot be denied that Thebesian channels may function as blood vessels, no experiments thus far reported have adequately demonstrated that Thebesian vessels operate as a blood supply or blood drainage system with the heart beating *in situ*. It is held that much of the evidence for Thebesian function obtained with other preparations is either not valid or does not apply to the heart beating *in situ* because of the unnatural preparations used, the failure to separate adequately superficial venous flow (particularly that from the anterior cardiac veins) from "Thebesian flow," and the failure to appreciate the existence of alternate venous routes by way of the inter- and extracardiac anastomotic connections. The extent to which the Thebesian channels function with the heart beating *in situ* cannot be determined until more exacting quantitative measurements are made of total coronary inflow and total superficial venous outflow (including flow in the extracardiac collaterals).

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COMBINATION OF EVANS BLUE WITH PLASMA PROTEIN: ITS SIGNIFICANCE IN CAPILLARY PERMEABILITY STUDIES, BLOOD DYE DISAPPEARANCE CURVES, AND ITS USE AS A PROTEIN TAG[1]

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Received for publication January 18, 1947

The use of isotopes has proved to be an important tool in the study of the metabolism of body constituents (1). The employment of such tracer substances has developed an awareness for easily identifiable substances which can be combined with a particular body constituent, in order to tag it for study purposes. It is understandable, therefore, that the announcement by Rawson(2) that Evans blue dye (T-1824) combines selectively with the albumin of blood plasma would foster studies employing this dye as a tracer for serum albumin. Thus, for example, Cope and Moore (3) studied the problem of capillary permeability to albumin in burn shock utilizing radioactive dibromo Evans blue as a tracer for albumin.

Evans blue dye (T-1824) has come into wide clinical use as a dyestuff suitable for determining blood volume. Gregersen and Rawson (4), in studies of the disappearance of T-1824 from the blood of dogs, observed that there was an early logarithmic phase of disappearance lasting for approximately one hour. This was followed by a linear phase during which time the dye disappeared from the blood at a constant rate. In view of Rawson's (2) demonstration that the dye combines selectively with albumin, the logarithmic phase of disappearance was considered as a measure of capillary permeability to albumin. Accordingly, its disappearance after the logarithmic phase would possibly mean that a definite quantity of albumin has been removed with the dye from the plasma. Since plasma protein concentration is maintained within narrow limits, that amount of dye-protein removed would have been replaced by protein uncombined with dye. From these considerations, the definite possibility exists that the rate of disappearance of dye from the blood stream would mirror the rate of albumin turnover in the body. The potentialities of this attractive idea for the development of a clinical test for quantitating albumin regeneration rates prompted us to explore this concept experimentally.

It is known that basic dyestuffs accumulate in high concentrations in the juice of actively secreting stomachs (5, 6) and acid dyestuffs, in the alkaline pancreatic juice. In these studies it has usually been considered that it was the permeability to the dye molecules alone which was being investigated. However, in the case of Evans blue, one would be following the behavior of a dye-protein complex, whose chemical and physical properties are necessarily different

[1] Supported by a grant from the Otho S. A. Sprague Memorial Institute.

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from the dye and protein molecules in the uncombined state. Our present information about the exact nature of the Evans-blue dye-albumin linkage or of its stability *in vivo* is very inadequate.

Accordingly, a critical study has been made of the significance of the disappearance of T-1824 from the blood stream, its appearance in gastric and pancreatic juices and the chemical nature of the dye-protein linkage.

Twenty-four-hour disappearance of Evans blue from the blood. Fasting blood specimens from patients and normals were drawn over a 24-hour interval beginning 4 hours following the injection of 10 cc. of 0.15% Evans blue and continuing

TABLE 1. *The disappearance of Evans blue dye from the blood of normal and of hypoproteinemic subjects*

PATIENT NUMBER	CLINICAL DIAGNOSIS	PLASMA PROTEINS	PERCENTAGE OF DISAPPEARANCE IN 24 HOURS
		grams/100cc.	
N-1	Normal	7.68	50
N-2	Normal	7.21	40
N-3	Normal	7.32	48
N-4	Normal	7.26	42
N-5	Normal	7.57	74
9	Ca. of stomach	6.26	38
2	Ca. of pancreas	6.64	23
12	Colostomy	6.58	27
11	Ca. of pancreas	6.53	35
10	Ca. of stomach	7.78	35
16	Ca. of stomach	5.26	58
13	Cardiospasm	6.70	44
5	Cirrhosis	6.49	49
1	Ca. of stomach	5.75	52
18	Ca. of stomach	6.14	61
17	Ca. of stomach	4.95	54
19	Ca. of stomach	6.02	74
3	Ca. of esophagus	5.74	33

to 28 hours following the injection of the dye. As a rule, 4 blood specimens were taken during this period.

The percentage of disappearance of Evans blue from the plasma was calculated from the 24-hour dye-disappearance curve. From table 1 it is apparent that there are no significant differences in the percentage of disappearance in normals and in hypoproteinemics. Also both groups show wide variations. The average percentage disappearance for the entire group was 46.

From the evidence presented, one is inclined to believe that the percentage of dye disappearance in 24 hours has little or no significance insofar as it may mirror albumin turnover, although the possibility cannot be excluded that perhaps 50% of the circulating albumin may be turned over in a 24-hour period. In the latter case, it would follow that in hypoproteinemia there is no difference from normal in the rate of protein turnover.

Appearance of Evans blue in gastric and pancreatic juice. In the course of another study on some dogs with Pavlov pouches and some with pancreatic fistulas, blood volumes were determined with T-1824. On some of these dogs the dye appeared in the secretions. These experiments were repeated to exclude the possibility that the dye was oozing from granulating surfaces. With an intravenous dose of 15 mgm. no dye appeared in the aspirated stomach contents of dogs either before or after the administration of histamine. In three dogs given an intravenous dose of 25 mgm. of Evans blue, the pancreatic ducts were canulated and juice collected after stimulation with secretin. No dye appeared in the pancreatic juice. In a similar experiment on one dog, 250 mgm. of the dye was given. The dose was sufficient to make the ratio of dye to serum albumin approximately 5. A definite blue tint was seen in the pancreatic juice. The fact that the dye appeared in the secretion of this one dog, even though in minimal concentration, was considered significant. It would seem that if the dye is given in great enough concentration it will appear in the pancreatic juice.

Chemical studies. The purpose of the chemical studies was to measure the extent of the combination of dye with individual plasma proteins. After many preliminary trials conditions were found which enabled this to be done quantitatively. Purified human plasma protein fractions[3] were mixed with varying concentrations of Evans blue. The dye-protein combination was then precipitated with trichloracetic acid and the excess dye remaining in the solution was read in an Evelyn photoelectric colorimeter.

The use of trichloracetic acid has the disadvantage, for purposes of comparison with the work of others (2), that the precipitation is carried out at an acid pH. However, it was found to be free of the more serious drawbacks of other protein precipitants which were tried, such as heavy metals which precipitate Evans blue, and methyl alcohol or $(NH_4)_2SO_4$ which did not precipitate the dye-protein quantitatively. Although the amount of dye which combines with protein at different pH may vary, the essential nature of the dye-protein linkage will remain unaltered. Accordingly, all precipitations have been done with trichloracetic acid.

A. Combination of Evans blue with purified albumin. Varying concentrations of albumin in normal saline were prepared so that 3 cc. contained from 0 to 2.6 mgm. of albumin. These albumin solutions were added to 6 cc. of 0.03% Evans blue buffered at pH 2.5 in a test tube and mixed by inversion. After a 10-minute interval (even though equilibrium is reached almost instantly), precipitation of dye-protein was accomplished by the addition of 5 cc. of 20% trichloracetic acid. The precipitated dye-protein was separated from the solution by centrifugation. (Filtration is unsatisfactory because the dye is absorbed by the filter paper.) The supernatant was transferred to an Evelyn tube, diluted 1:20 with distilled water, and then read in an Evelyn photoelectric colorimeter using a no. 620 filter. The density of the supernatant dye is plotted in figure 1. The determinations were made in triplicate and the data on figure 1 have been reproduced on other occasions.

[3] Purified human plasma proteins were obtained through the courtesy of Dr. E. J. Cohn of Harvard University Serum Laboratory.

Since the plot is linear only when a great excess of dye is present and tends to level off and reach a plateau with relatively high protein concentration, a characteristic of dissociation curves of weak acids and bases, one may reasonably assume that the reaction is reversible.

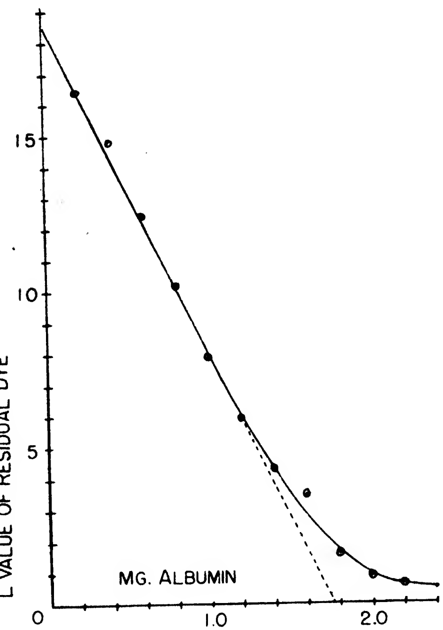

FIG. 1

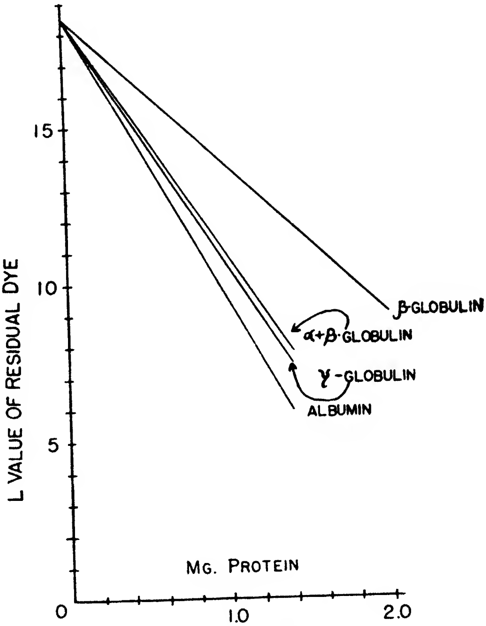

FIG. 2

FIG. 1. The combination of varying concentrations of purified human serum-albumin with Evans blue dye is demonstrated. The dotted straight line is the expected curve with an irreversible reaction (dye + protein → dye-protein). Single determinations are represented by dots, coinciding duplicate and triplicate determinations by circles. All determinations were made in triplicate. (Optical density is expressed as 'L values'.)

FIG. 2. The combination of varying concentrations of purified human plasma-protein fractions with Evans blue is indicated. Only the linear portions of the curves have been represented in each case. (Optical density is expressed as 'L values.')

$$\text{dye} + \text{protein} \rightleftarrows \text{dye-protein}$$

In this case, the law of mass action should apply according to the following equation: $\frac{(\text{dye})\ (\text{protein})}{(\text{dye-protein})} = \text{K}$. The linear portion of the curve represents the stoichiometric combination of the dye with protein, since the reversible reaction has been displaced to the right by an excess of dye. Under these conditions it can be calculated that 70 moles of dye combine with one of albumin. Let us assume in this experiment that approximately 70 moles of dye are combined with protein on the non-linear portion of the curve in figure 1; then the initial concentrations of dye and protein are known and the concentration of dye-protein can be calculated by the difference from the residual dye. Molar concentrations

are obtained by dividing the gram concentrations by the molecular weight of purified albumin (70,000) and dyestuff (960), respectively. When such calculations are made for several points on the non-linear portion of the curve, the following constant is obtained: 2×10^{-3}. If one presumes that the combination of the dye with protein is the same in both its dissolved and precipitated phase, this figure (2×10^{-3}) represents the dissociation constant.

B. Combination of Evans blue with globulins. Experiments similar to the above were performed with purified human-plasma-globulin fractions. The combination of T-1824 with the various globulin fractions as compared to albumin is pictured in figure 2. The globulins fix less dye than albumin, since the slopes of the linear portions are less steep. Certainly under these conditions Evans blue dye does not combine specifically with albumin. When various known mixtures of albumin and globulin are precipitated with trichloracetic acid in the presence of T-1824, the density of the residual dye is always less than would be expected with albumin alone, indicating that globulin takes up some of the dye.

C. Combination of Evans blue with bovine plasma-protein fractions. When highly purified bovine plasma-proteins[4] are substituted for human plasma-proteins, similar curves as in figures 1 and 2 are obtained. Quantitatively the extent of the combination of dye with bovine or human albumin is identical. There are some individual differences with the bovine globulins as compared to human globulins, but they both combine with dye to approximately the same degree.

D. Combination of Evans blue with cellophane and exchange resins. Cellophane was stained with a concentrated solution of T-1824. The stained cellophane was washed first with repeated changes of distilled water for a period of two weeks and then with repeated changes of normal saline for a similar period. After a short interval the washings were no longer colored by dyestuff. The stained and washed cellophane was then transferred to a 5-gram % albumin solution. Color immediately left the cellophane and was seen in the protein solution. The cellophane had acted in a fashion similar to an ion exchange resin.

A 5½-inch resin column which contained approximately 5 grams of Dualite A-2 (RCl form) was set up. Seven cc. of a 0,05% Evans blue solution containing 5 mgm. per 1 cc. of albumin (ratio of dye to albumin = 7.29) was run through the column at a rate of approximately 1 cc. per minute. Fifty % of the dye was removed by the resin, although the protein was recovered quantitatively. Since the insoluble resin accepts only free dye anions, dissociation of the dye-albumin complex must have occurred. The dye could not be washed from the resin by distilled water but was liberated by sodium hydroxide which changed the resin from the R-dye form to the R-OH form.

Discussion and conclusions. The evidence of Rawson that Evans blue dye attaches itself to albumin is based mainly on electrophoretic data, on ultracentrifugation experiments and on spectrometric studies. It was found that

[4] Purified bovine plasma fractions were obtained through the courtesy of Armour and Company, Chicago, Illinois.

Evans blue in serum migrated exclusively with the albumin fraction except with a high concentration of dye, at which time some dye migrated with α globulin (2). These results have been confirmed by others (7). Such studies do not show the exact nature of the binding, that is, whether it is chemical or physical in nature.

Our chemical studies are explained best on the basis that T-1824 forms a dissociable compound with plasma albumin and globulin. Others (8) like ourselves have found that dye-protein combination follows the law of mass action and that dissociation constants can be derived. The reaction is not specific for albumin but selective at certain concentration. The dissociation constant is an average for the many groups that combine with dye forming a dye-protein complex. On the basis that dyestuffs titrate acid and basic groups in the protein molecule (9), our findings agree with other observations (10). Also, since Evans blue is an acid dye, the maximum amount of dyestuff will combine with the protein when it is entirely in its basic form, i.e., at a very acid pH. Maximally 70 moles of Evans blue combine with one of albumin. At neutrality, fewer moles of dye would combine with albumin. This explains the discrepancy between our findings and those of others (2).

Careful studies have led to the concept that ions as such are generally not able to permeate cell membranes (11, 12). Since Evans blue occasionally appears in pancreatic juice, one must surmise, at least that the unionized dye molecule is capable of diffusing past the capillary membrane. According to present day concepts, this means that the dye is trapped by ionization (5, 6) in the secreted juice. These authors and many others have observed that neutral red, which is a plasma protein-binding dye, appears in high concentrations in acid gastric juice. Trypan blue, also plasma-binding, appears in pancreatic juice. These observations are most readily explained on the basis of a dissociable dye-protein complex existing *in vivo* and do not indicate any great or unique stability of this linkage.

That tissue protein also fixes dye is evident from autopsies on dogs which received Evans blue. The lymph nodes are always more deeply stained than other tissues (13). As the dye diffuses from the circulation and is fixed in the tissues, more dye-protein is dissociated to maintain chemical equilibrium.

One may therefore hypothesize the following conditions as occurring *in vivo:*

Dye-protein ⇄ unionized dye + protein (blood)

↓↑ (capillary membrane)

unionized dye (tissue fluids)

↙ ↘

trapping in some secretions by ionization

Fixation of dye by tissue protein.

Because little dye is in the free form in those concentrations obtained in the blood clinically, the diffusion of these free dye molecules into the interstitial

fluid would not completely account for the early rapid phase of disappearance from the blood. It is evident from the scheme that the fixation of the free dye molecules by tissue protein would cause more dye to leave the blood until the tissue proteins become saturated. This may explain the early rapid phase of disappearance. Such an interpretation would be in agreement with the findings of Fine and Seligman (14, 15), who found no rapid initial disappearance of infused radioactive plasma protein. The duration of the 'logarithmic' phase of disappearance of protein-binding dyes would probably be determined by the degree of dissociation of the dye-protein complex. The more highly dissociated the complex, the more rapidly would a steady state be established, bringing about a short initial phase. It would follow that the extent of dyestuff disappearance in the 'logarithmic' phase would depend upon the amount of dye the tissue proteins are capable of fixing. Apparently, the body can do quite easily what we attempted to do *in vitro* with the dye-protein ion-exchange resin experiment. Others (16) have approached the problem in a manner different from ours, but, like ourselves, have concluded that T–1824 is fixed to tissue protein during the early phase of blood dye disappearance.

To what extent then does Evans blue measure the capillary permeability to albumin? In those blood dye concentrations obtained clinically, Evans blue exists almost exclusively in the form of dye-protein complex because of the excess molecular concentration of protein which displaces the reversible reaction to the right. Therefore Evans blue appears wherever plasma protein is present. Since the dye-protein combination is reversible, and since it can diffuse in its free form, dye may migrate and slowly become fixed in sites where serum protein fails to appear. In other words, when albumin migrates (as into a burned area), Evans blue must follow. The converse is true only if one can show that there is no fixation by other tissue protein or no ionization of the free dye molecules. The initial disappearance of Evans blue from the bloodstream apparently does not mirror the capillary permeability to albumin.

Should not the dye be a good tag for albumin in metabolic studies even though it may not measure capillary permeability to albumin? The late linear disappearance phase of Evans blue dye from human plasma over a 24-hour period amounts to approximately 50% of the dye concentration. This should mean that 50% of the circulating albumin had been turned over in a 24-hour period. Quantitatively such figures are incompatible with the better isotopic studies of others (14, 15). The rapid disappearance of dye in contrast to the relatively slow rate of disappearance of isotopically tagged plasma protein is readily explained on the basis that dye can be dissociated from the albumin molecule without the latter being metabolized. Once the dye has been removed from the protein molecule, it may return to the circulation and recombine with protein or be lost in metabolism, fixation or excretion. With the isotopic studies the isotopic element is an integral part of the protein molecule, which must be catabolized before the isotope is released. Evans blue or other dyestuffs therefore can not be used as a tag for protein in metabolic studies.

SUMMARY

Evans blue dye (T-1824) forms a dissociable complex with plasma protein in the present experimental conditions. The dye-protein combination is not specific for albumin, since the dye combines readily with all the globulin fractions. With those blood dye concentrations obtained in the clinical use of Evans blue, most of the dye is probably united to albumin.

The use of Evans blue as a label for protein in studies of capillary permeability and protein metabolism has been critically discussed and its limitations have been pointed out. Because the dye protein compound is dissociable and because the dye is not an integral part of the protein molecule, its clinical use as a tracer for protein becomes limited.

In vitro observations and studies with humans and dogs have provided a basis for interpreting the disappearance and appearance of Evans blue dye in tissue fluids.

Gratitude is expressed to Dr. D. B. Phemister whose active interest made these studies possible. We are indebted to Dr. R. K. Cannan who suggested the ion exchange resin experiment. Thanks are also expressed to Mrs. Evelyn Gordon and Mr. Lamarr Walker for their clinical help.

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EFFECTS OF MUSCLE TRAUMA AND OF HEMORRHAGE UPON THE CARDIAC OUTPUT OF THE DOG[1]

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Received for publication July 26, 1947

After traumatic injury the clinical signs of shock develop gradually, and up to the time of death there is increasing evidence of progressive failure of an adequate blood supply to the tissues. The limbs grow colder, the body temperature drops, the mucous membranes of the mouth become increasingly pale and dry, the venous blood becomes darker in color and eventually the animal shows signs of central nervous depression. Nevertheless, we have been unable to find that the changes are associated with a progressive reduction in blood volume (1). To be sure, the blood volume falls 30 to 50% at the time of trauma or immediately thereafter, but from then on it undergoes no further change. For this, we have evidence not only that the plasma volume as measured with the dye T-1824 remains essentially unchanged up to the time of death, but also that neither the serum protein concentration nor the hematocrit values are significantly altered. The progressive nature of the symptoms in the presence of an unchanging blood volume clearly indicates that certain undetermined circulatory changes must be taking place. The rising heart rate, the increasing difficulty with which blood can be drawn from a peripheral vein and the increasing difference in color between arterial and venous blood which we have consistently observed in shocked dogs suggest that progressive changes in venous return, cardiac output and oxygen consumption may account for the gradual circulatory failure.

Several investigations upon anesthetized animals indicate that during shock the circulatory and metabolic changes mentioned above do occur. For example, intestinal manipulation (2) and muscle trauma (3) are said to produce a large decrease in the oxygen content of the venous blood. Moreover, it is known that the oxygen consumption and the cardiac output are reduced by muscle trauma (3, 4, 5). In the present investigation (May and June, 1942), we measured the cardiac output before and at various times after the production of shock by muscle trauma. These experiments were compared with others in which the blood volume was reduced by bleeding.

METHODS. The animal was placed upon its back on an animal board. During the control period the arterial blood pressure (arterial puncture, Hg manometer), the heart rate, the rectal temperature and the cardiac output were estimated. Muscle trauma was carried out according to the procedure described by Gregersen and Root (1), or the animal was bled through a cannula inserted

[1] This work was done under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

in the femoral artery. At various times thereafter the above determinations were repeated and observations made of the coldness of the extremities, the blanching of the oral mucous membranes and the degree of central nervous depression.

In most of the animals the plasma volume was determined before and after the blood volume reduction. For this purpose the blue dye, T-1824, was used, optical densities being estimated spectrophotometrically (1). The blood volume was calculated from the plasma volume and the hematocrit reading. It has recently been shown that this method of calculating blood volume agrees to within 5 to 10% with that obtained by measuring plasma volume with T-1824 and red cell volume with CO (6).

The cardiac output was measured by the direct Fick method. Expired air was collected in a Douglas bag and analyzed for O_2 and CO_2 with the Haldane-Henderson gas analyzer. To avoid gas leakage the expired air was collected through a tracheal cannula which was inserted under local anesthesia (2% novocain). Heart rates were counted in 10 dogs during the insertion of the tracheal cannula and found to range between 53 and 84 beats per minute, indicating that this procedure disturbs the animals little if at all. In some experiments the O_2 consumption, the CO_2 production and the R.Q. were measured with Scholander's respirometer[2] (7). In this method the animal produces a graphic record (see fig. 1) of respiratory exchange by inspiring from the top of an O_2-filled spirometer system, the expired air being introduced without absorption of CO_2 into the bottom of the system where it is present as a gas layer of greater density than O_2. The slope of the respirogram indicates the magnitude of the apparent R.Q., sloping downward when this is less than 1.0, being level when the ratio is equal to 1.0 and sloping upward when the R.Q. is greater than 1.0. At the end of 5 minutes the expiratory tubes are switched to a second spirometer system. The previously expired gas is now circulated through alkali, the curve of CO_2 absorption being recorded graphically. When the second 5-minute period is completed the dog is reconnected to the spirometer first used which now has been freed of CO_2 and refilled with O_2. In this way continuous records are obtained of the apparent R.Q. and CO_2 production from which the O_2 consumption can be calculated. The periods of CO_2 absorption indicate time in 5-minute blocks. Arterial and mixed venous blood samples were taken immediately before and at the end of the period during which the expired air was collected in the Douglas bag or at the beginning and at the end of selected periods on the Scholander respirometer records.

Arterial blood samples were drawn under oil from the femoral artery, heparin being used as anticoagulant. Mixed venous samples were obtained from the right heart by means of a catheter inserted through the left external jugular vein. When the tip of the catheter enters the heart the impact of the systolic contraction upon the catheter is easily recognized. Clot formation was prevented by the slow infusion of saline at a rate of 1 to 2 cc. per minute. The

[2] We are indebted to Drs. L. W. Irving and P. F. Scholander for the loan of and instruction in the use of the Scholander respirometer.

withdrawal of the mixed venous blood sample was preceded by the removal of 7 to 10 cc. of the blood saline mixture in order to avoid dilution of the sample. The mixed venous sample was then taken under oil, using heparin as anticoagulant. The blood saline mixture was returned to the circulation. Blood O_2 content was determined on 1 cc. samples with the manometric Van Slyke apparatus. In 90 % of the determinations the O_2 contents of the samples obtained

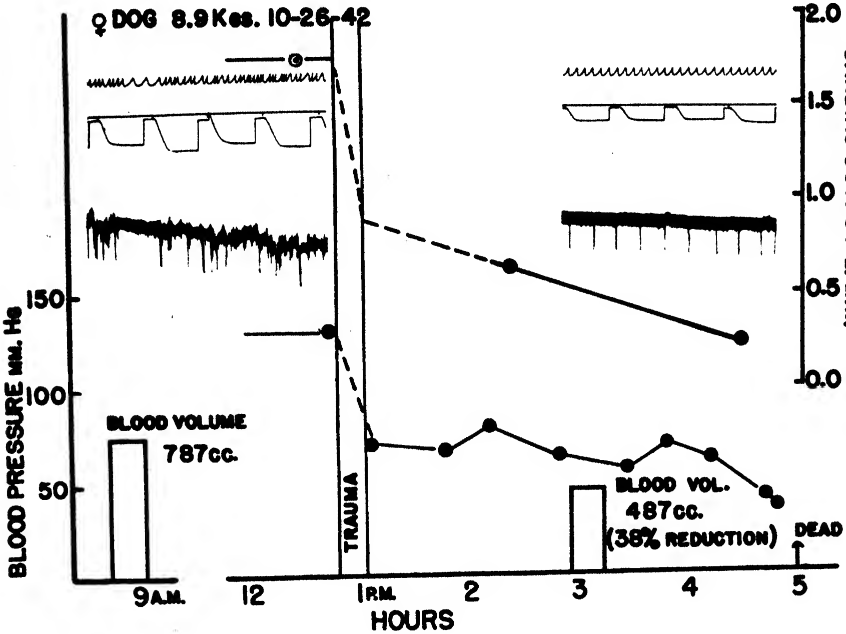

Fig. 1. *The effect of muscle trauma upon dog 9 (table 1).* The progressive decrease in cardiac output following a 38% reduction of the blood volume is illustrated. The inserts show the control respiratory record and that obtained 206 minutes after muscle trauma as measured with the Scholander respirometer. In the control record the slope of the curve of the individual respirations indicates the amount by which the rate of O_2 consumption is greater than the rate of CO_2 production. Note that this curve is less steep in the post-traumatic measurement indicating a higher apparent R.Q. Above the graph of individual respirations the curve of CO_2 absorption is shown. This tracing indicates time in 5-minute periods. Note the large decrease in CO_2 production in the post-traumatic tracing.

before the expired air collections agreed with those obtained at the end of the collection to within 1.5 volumes %, the average being 0.7 volume %. The values given in the tables are the average of these 2 determinations. According to Shore, Holt and Knoefel (8) the O_2 content of blood frequently differs when drawn simultaneously from the 2 vena cavae, from 2 points in the right auricle, or from the right auricle and ventricle of dogs anesthetized with barbital. Although in our experiments we have no direct evidence of the exact position of the tip of the catheter in the heart, the changes in the O_2 content of the mixed

venous blood are so great after hemorrhage or muscle trauma that variations of the magnitude reported by Shore *et al.* (8) would have relatively little effect upon our results.

RESULTS. Repeated determinations of the cardiac output were made upon 2 normal dogs (experiments 1 and 2, table 1). These show that over a period of 4 to 5 hours the variations in cardiac output are of the order of 10 % of the control value. Control estimations of the cardiac output were carried out upon each of the 28 animals studied (tables 1 and 2).

Muscle trauma. The effects of muscle trauma upon the various respiratory and circulatory measurements made in this investigation are shown in table 1. In 4 dogs (experiments 3 to 6, table 1) the injury was not severe enough to produce shock, as determined by the criteria used by Gregersen and Root (1). Well-defined shock developed in 10 animals (experiments 7 to 16, table 1). All the shocked dogs died between 1 hour and 37 minutes and 5 hours and 13 minutes after injury. No doubt some of these animals would have lived longer if blood samples had not been drawn from time to time.

The expired air was collected in 14 experiments using a Douglas bag. The average control pulmonary ventilation was about 5 liters per minute. During the first hour after traumatic injury the respiratory volume was always greater than the control value, averaging 10 liters a minute. Although the volume of the expired air decreased as shock developed (average 8 liters), it did not fall below the control level. Less pronounced changes were shown by those dogs in which muscle injury did not produce shock.

Inspection of table 1 shows that already 30 to 50 minutes after injury the O_2 consumption of all the shocked dogs had fallen considerably. After this time the metabolic rate continued downward but more gradually. When trauma was not severe enough to produce shock the effect upon the O_2 consumption was variable.

The low apparent respiratory quotients (R.Q.) shown in the control measurements on 25 of the 28 dogs studied were probably obtained because the animals were not fed during the preceding 24 hours. In every instance the R.Q.s of the dogs in traumatic shock (experiments 7 to 16, table 1) were greater than those measured during the control periods. When the severity of the trauma was insufficient to produce shock, the increase in the post-traumatic R.Q. was usually less than that shown by the shocked dogs.

After muscle trauma the arterial O_2 content varied in different animals between a small decrease and a slight increase as compared with the control values. Such changes are probably related to variations in the number of red cells contained in the blood reservoirs. The O_2 content of the mixed venous blood, however, was greatly reduced. The decrease occurred within 30 minutes after injury and unless recovery took place the venous blood became more and more reduced. Muscle trauma which was not severe enough to produce shock resulted in smaller reductions in the O_2 content of the mixed venous blood. The arterial-mixed venous O_2 difference increased greatly after trauma. Indeed, in one animal (experiment 11, table 1) the blood returning to the heart was completely reduced.

TABLE 1. *The effect of muscle trauma upon the cardiac output of dogs*

NO.	WT.	REDUCTION BLOOD VOL.	TIME AFTER TRAUMA	EXP. AIR	O_2 CONSUMED	R.Q.	A-V O_2 DIFF.	CARDIAC OUTPUT	STROKE VOL.	R	METHOD	REMARKS
	kgm.	*%*	*min.*	*L./min.*	*cc./min.*		*vols. %*	*L./min.*	*cc.*			
1	11.6	0	0	5.5	109	0.78	4.5	2.42	28.8	3400	D.B.	control—no trauma
			+83	5.7	112	0.78	4.3	2.60	18.3	3700		
			+257	3.5	100	0.70	3.6	2.78	20.1	3100		
2	12.0	0	0	2.8	87	0.70	4.2	2.07	39.0	5000	D.B.	control—no trauma
			+104	3.1	95	0.68	4.3	2.21	36.8	3700		
			+208	3.1	93	0.66	5.0	1.86	33.2	4800		
			+293	3.0	92	0.70	5.0	1.80	27.7	4700		
3	12.4	16	−29	2.7	88	0.66	6.2	1.42	20.5	5400	D.B.	trauma—no shock; survived
			+67	3.8	90	0.73	6.4	1.41	9.3	6400		
			+202	3.3	89	0.71	5.8	1.53	10.7	6100		
4	11.8	17	−45	2.2	83	0.65	5.9	1.41	21.6	6700	D.B.	trauma—no shock; survived
			+43	2.9	82	0.81	9.3	0.88	5.8	9700		
			+226	3.3	78	0.80	11.7	0.67	4.0	12400		
			+295	3.2	84	0.82	11.1	0.76	8.4	10600		
5	14.8	24	−38	8.3	164	0.95	3.3	4.97	42.1	1700	D.B.	trauma—no shock; survived
			+54	11.9	112	0.83	5.1	2.20	16.6	3500		
			+200	4.4	100	0.90	4.2	2.38	21.2	3400		
			+289	3.3	78	0.99	6.1	1.28	11.5	6500		
6	11.7	25	−39	3.7	119	0.76	4.9	2.43	28.9	2800	D.B.	trauma—no shock; survived
			+51	4.8	86	0.87	5.9	1.46	9.0	3200		
			+193	4.0	77	0.82	6.7	1.15	6.2	5000		
7	7.4	37	−41	—	105	0.71	4.2	2.50	20.8	3700	S.R.	trauma—shock; died 4 hrs. 44 min.
			+144	—	56	0.88	13.6	0.41	2.9	11800		
			+217	—	51	0.84	12.9	0.39	2.4	10300		
8	10.3	38	−59	5.7	122	0.73	5.9	2.07	19.1	4500	D.B.	trauma—shock; survived
			+50	17.1	56	0.94	9.4	0.60	2.9	9800		
			+125	7.1	56	0.88	11.4	0.49	2.9	10800		
9	8.9	38	−18	—	120	0.75	6.9	1.74	11.6	6000	S.R.	trauma—shock; died 4 hrs. 51 min.
			+20	—	84	0.67	13.0	0.64	3.2	8800		
			+206	—	43	0.94	17.7	0.24	1.2	16700		
10	10.1	46	−39	4.9	98	0.75	66.3	1.56	11.3	4600	D.B.	trauma—shock; died 1 hr. 46 min.
			+69	6.8	36	1.00	10.5	0.34	2.2	8200		

TABLE 1—*continued*

NO.	WT.	REDUCTION BLOOD VOL.	TIME AFTER TRAUMA	EXP. AIR	O_2 CONSUMED	R.Q.	A-V O_2 DIFF.	CARDIAC OUTPUT	STROKE VOL.	R	METHOD	REMARKS
	kgm.	*%*	*min.*	*L./min.*	*cc./min.*		*vols. %*	*L./min.*	*cc.*			
11	13.1	—	−44	3.5	129	0.64	3.2	4.02	30.6	1900	D.B.	trauma—shock; died 2 hrs. 18 min.
			+84	8.8	77	0.87	14.5	0.53	2.8	6100		
12	12.1	—	−43	3.3	98	0.71	2.5	3.92	32.6	2200	D.B.	trauma—shock; died 4 hrs. 32 min.
			+80	4.9	78	0.79	11.4	0.68	4.1	6600		
			+148	5.4	66	0.89	14.2	0.46	2.7	10300		
			+220	7.1	63	1.00	15.3	0.41	2.1	6500		
13	11.2	—	−55	6.4	125	0.68	3.5	3.57	26.4	3100	D.B.	trauma—shock; died 2 hrs. 6 min.
			+37	16.3	76	0.87	17.3	0.44	2.3	1000		
			+103	10.4	60	1.05	22.3	0.27	1.4	14200		
14	14.1	—	−45	17.1	183	0.71	7.0	2.62	37.9	3400	D.B.	trauma—shock; died 5 hrs. 13 min.
			+41	22.7	113	1.00	15.7	0.72	4.2	6700		
			+155	14.3	110	0.93	12.0	0.92	4.8	5900		
			+270	7.6	80	0.99	19.1	0.42	1.9	10200		
15	12.1	—	−57	3.4	114	0.67	5.4	2.10	25.9	4000	D.B.	trauma—shock; died 1 hr. 56 min.
			+45	7.1	58	0.95	16.6	0.35	1.9	12700		
			+105	5.3	27	0.99	17.1	0.16	0.8	15800		
16	9.4	—	−31	5.7	123	0.71	4.4	2.80	16.6	3300	D.B.	trauma—shock; died 1 hr. 37 min.
			+51	6.2	71	0.82	14.5	0.49	2.6	10600		

$$R = \frac{\text{mean blood pressure}}{\text{cardiac output/sec.}} \times 1332.$$

D.B. = Douglas bag technique.

S.R. = Scholander respirometer.

Time = 0 is the start of the infliction of muscle trauma; negative values refer to time before this.

The cardiac output decreased progressively after muscle trauma (experiments 7 to 16, table 1). The output per beat decreased to an even greater extent. Smaller changes in these values were shown by traumatized dogs which did not develop shock (experiments 3 to 6, table 1).

TABLE 2. *The effect of hemorrhage upon the cardiac output of dogs*

NO.	WT.	REDUCTION BLOOD VOL.	TIME AFTER TRAUMA	EXP. AIR	O_2 CONSUMED	R.Q.	A-V O_2 DIFF.	CARDIAC OUTPUT	STROKE VOL.	R	METHOD	REMARKS
	kgm.	*%*	*min.*	*L./min.*	*cc./min.*		*vols. %*	*L./min.*	*cc.*			
1	13.7	11	−55	4.6	115	0.78	2.5	4.60	33.3	2500	D.B.	Survived
			+10	4.1	87	0.78	7.0	1.24	16.8	3900		
			+208	3.7	102	0.76	4.8	2.13	14.0	5000		
2	11.0	21	−76	1.6	66	0.70	7.3	0.90	17.0	11000	D.B.	Survived
			+18	2.4	74	0.70	7.8	0.95	7.2	10000		
			+156	2.3	76	0.85	6.8	1.12	6.2	9000		
3	11.2	24	−73	3.1	81	0.71	4.6	1.76	16.7	5000	D.B.	Survived
			+8	2.9	77	0.74	5.7	1.35	7.7	4000		
			+201	3.5	69	0.79	7.4	0.93	5.8	6900		
4	13.0	28	−84	7.5	135	0.77	2.8	4.82	37.3	2300	D.B.	Survived
			+23	8.4	92	0.79	9.3	0.99	6.6	5000		
			+207	6.1	81	0.92	8.6	0.94	6.4	7300		
5	15.3	34	−28	—	214	0.70	4.6	4.65	36.0	2200	S.R.	Survived
			+78	—	125	0.90	13.4	0.93	4.9	4100		
			+266	—	135	0.85	15.9	0.85	4.3	5700		
6	9.9	37	−15	—	158	0.94	5.1	3.09	21.6	2700	S.R.	died 4 hrs. 15 min.
			+10	—	32	1.09	16.2	0.20	1.3	10000		
			+77	—	76	0.91	15.7	0.48	2.8	7500		
			+139	—	86	0.81	11.9	0.72	3.7	7800		
			+175	—	70	0.86	13.5	0.52	2.4	8500		
7	13.0	43	−75	—	124	0.84	4.5	2.75	24.7	3900	S.R.	died 2 hrs.
			+95	—	82	0.99	12.5	0.69	3.2	4600		
8	11.4	43	−20	—	126	0.78	4.7	2.68	17.8	3800	S.R.	died 2 hrs. 1 min.
			+26	—	70	0.82	13.1	0.53	3.9	6800		
			+103	—	47	0.86	15.0	0.31	1.9	8400		
9	12.2	44	−33	—	176	0.67	5.5	3.20	33.3	2800	S.R.	Survived
			+28	—	94	0.78	11.1	0.85	5.0	4600		
			+178	—	103	0.86	14.2	0.72	2.9	5000		
10	9.9	47	−29	—	165	0.76	5.7	2.89	16.0	3200	S.R.	died 55 min.
			+39	—	51	1.01	14.7	0.35	2.5	6900		
11	11.6	47	−59	9.0	85	0.66	6.1	1.39	22.8	6800	D.B.	died 6 hrs. 45 min.
			+7	6.4	48	1.13	14.9	0.32	1.9	6800		
			+87	6.7	71	0.78	11.0	0.64	3.1	6300		
			+227	6.4	66	0.90	12.4	0.53	2.2	7600		
12	6.0	49	−34	—	79	0.71	4.3	1.83	9.3	4100	S.R.	died 1 hr. 8 min.
			+33	—	27	0.96	9.5	0.28	1.6	8100		

$$R = \frac{\text{mean blood pressure}}{\text{cardiac output/sec.}} \times 1332$$

D.B. = Douglas bag technique.
S.R. = Scholander respirometer.
Time = 0 is measured from the start of hemorrhage; negative values refer to time before start of hemorrhage.

The total peripheral resistance (R) was calculated, using the formula (see 9):

$$R = \frac{\text{mean blood pressure} \times 1332}{\text{cardiac output per second}} \text{ in } \frac{\text{dynes second}}{\text{cm}^5}$$

When traumatized dogs developed shock, the calculated total peripheral resistance increased 1–5-fold over the control values. Smaller increases in the R values were shown by dogs in which the injury was not severe enough to produce shock.

The blood volumes were measured before and after trauma in 8 dogs (experiments 3 to 10, table 1). In 4 of these (experiments 3 to 6), which failed to develop shock, the blood volume reduction ranged between 16 and 25 %. The usual signs of severe shock were shown by the 4 animals in which the blood volume was reduced from 37 to 46 %. These results are in agreement with those obtained previously in this laboratory (1).

Some of the data obtained on *dog 9* (table 1) are shown graphically in figure 1. The blood volume of this animal was reduced 38 % by muscle trauma. The procedure resulted in a decrease in mean arterial blood pressure which remained between 50 to 70 mm. Hg for more than 3 hours. During this period the cardiac output fell from a control of 1.74 to 0.84 liters per minute 20 minutes after trauma, and to 0.24 liter 3 hours and 6 minutes later. The insert shows the respiratory record obtained with the Scholander respirometer. In the control period the tracing of the individual respirations indicates an R.Q. of 0.75. The O_2 consumption was 120 cc. per minute. Three hours and 26 minutes after injury the respiratory tracing is flatter, indicating a high R.Q. and the curve of CO_2 absorption is much smaller than in the control period. The O_2 consumption has now decreased to 43 cc. per minute.

Hemorrhage. Twelve unanesthetized dogs were bled amounts ranging from 11 to 49 % of their control blood volumes. The results are summarized in table 2.

The cardiac output decreased immediately after bleeding. In some dogs the value returned toward the control levels (experiments 1, 2, 6, and 11, table 2) whereas, in others the output continued to decrease (experiments 3, 4, 5, 8 and 9, table 2).

After hemorrhage the heart rate was usually more rapid than during the control period. This was not the case in experiments 1 and 8 (table 2), but these dogs were excited, the control heart rates lying between 140 and 200 beats per minute. Rapid, post-hemorrhagic heart rates were associated with stroke volumes which had decreased to a smaller fraction of the control stroke volumes than had the corresponding cardiac outputs. After bleeding, the total peripheral resistance (R) increased 1- to 3-fold in 11 of the 12 dogs studied.

No marked changes in the expired air volumes were observed after hemorrhages amounting to 11 to 28% of the control blood volumes. By chance the pulmonary ventilation was not measured in the animals in which the blood volume was reduced more than 30%.

The O_2 consumption decreased considerably immediately after hemorrhage.

Subsequent determinations showed that the O_2 consumption continued to decrease in some experiments (*dogs 2, 3, 4* and *8*, table 2). In other animals (*dogs 1, 5, 6, 9* and *11*) the initial fall in the metabolic rate was followed by a variable amount of recovery. In most of the experiments a higher R.Q. was found after hemorrhage than during the control period.

The arterial O_2 content was not greatly altered by the bleeding, whereas in all but one instance (experiment 2) the O_2 content of the mixed venous blood decreased considerably. The arterial-mixed venous O_2 increased, the maximum increases occurring in the dogs which were subjected to the largest hemorrhages.

Discussion. Even the most casual reader must be impressed by the low cardiac outputs and the very small outputs per beat which are shown by our shocked animals. In this connection the recent statement of Eckstein *et al.* (10) that a blood flow of 150 cc. per minute is approximately the lowest critical level which sustains the heart and circulation of a 10 to 12 kgm. dog is of interest.

The decreased cardiac output which follows hemorrhage or muscle trauma markedly reduces the volume flow through the tissues. This is associated in our experiments as well as in those of Blalock and Bradburn (11) with a progressive decrease in the O_2 content of the mixed venous blood. The decrease in the mixed venous O_2 content (increase in arterial-mixed venous O_2 difference) is a function of the rate of blood flow and of the O_2 consumption of the tissues. Since in our experiments the change in arterial-mixed venous O_2 content is so much greater than the change in O_2 consumption the magnitude of the former can be used as an index of the decrease in cardiac output (fig. 2).

The fact that it is often impractical to obtain mixed venous blood for the determination of O_2 content makes the relation of the O_2 content of blood in the peripheral veins to that in the mixed venous blood of interest. According to the data of Blalock and Bradburn (11) the changes in arterial-femoral or jugular venous O_2 difference which follow muscle trauma or hemorrhage bear a linear relation to the arterial-mixed venous O_2 difference. A similar relation can be shown by plotting the arterial-jugular venous O_2 differences against the arterial-mixed venous O_2 differences of dogs injected subcutaneously with histamine (12) or by comparing the O_2 contents of portal and tail veins of bled rats (13). Gregory and Ewing (14) who studied the peripheral venous O_2 content of dogs subjected to muscle trauma found a progressive and large decrease in the venous O_2 content in all of the animals that died, but not in those which survived. According to these authors extreme decreases in the O_2 content of the venous blood may occur many hours before blood-pressure changes or before sudden death. It is, of course, well known that blood draining exposed regions of the body may vary greatly in O_2 content as compared with venous blood in deeper regions of the body (15). Nevertheless, we have found that the O_2 content of the jugular venous blood correlates well with other metabolic changes related to blood flow (16).

Although the hemodynamic changes produced by hemorrhage and muscle trauma are similar, certain differences are present. The arterial pressures and the peripheral resistances are higher in traumatized dogs than in animals in

which the blood volume has been reduced to the same extent by hemorrhage. Moreover, traumatized dogs rarely survive with a residual blood volume less than 65 cc. per kgm. of body weight, whereas in hemorrhagic shock animals do not succumb unless the blood volume is reduced to 58 cc. per kgm. (17). The clinical appearance of the animals in the two forms of shock is not the same, but the difference is difficult to define.

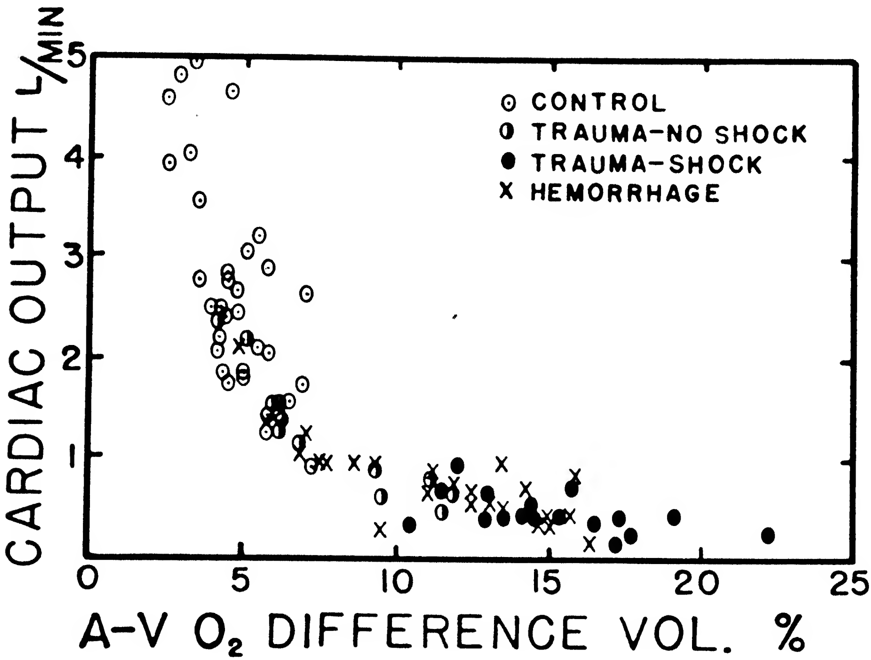

FIG. 2. *The relation of cardiac output to the arterial-mixed venous O_2 difference in dogs.*

SUMMARY

When the blood volume of dogs is reduced by 30 to 40% following muscle trauma, shock occurs. The O_2 consumption decreases to between 40 and 60% of the control values. The cardiac output (Fick method) is progressively reduced eventually reaching values only 10 to 25% of the control outputs. The high heart rates of severe shock are associated with extremely small outputs per beat (1 to 3 cc.). Arterial O_2 saturation is relatively unchanged, but the mixed venous O_2 content is low, being almost completely reduced in some instances. These changes in the venous blood greatly increase the A-V O_2 difference which sometimes approximates the O_2 capacity of the blood. The pulmonary ventilation and the R.Q. increase. Calculated peripheral resistance increases.

For the same reduction in blood volume the respiratory and circulatory changes which occur in bled dogs are similar to those observed following muscle trauma.

Certain differences are present, however, for the arterial blood pressures and the calculated peripheral resistances are higher in traumatized animals than in those in which the blood volume has been decreased by hemorrhage.

The relation of the peripheral venous O_2 content to the cardiac output is discussed.

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MEASUREMENT OF CARDIAC OUTPUT BY A CONTINUOUSLY RECORDING CONDUCTIVITY METHOD[1]

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Received for publication August 9, 1947

If a measured amount of a substance is rapidly injected into a vein, and its mean concentration in arterial blood during the period of its first passage past a collection point in an artery is determined, the cardiac output is given by the expression $F = \frac{60A}{TC}$, where F is cardiac output in liters per minute, A is the amount of the injected substance in milligrams, T is the number of seconds duration of the curve of passage and C is the mean concentration of the added substance, expressed as milligrams per liter, in the arterial blood during the T seconds time of passage (1, 2); this is procedure II of Stewart's 1921 paper. Stewart's technique did not permit a continuous recording of the conductivity changes resulting from rapid injection of hypertonic sodium chloride solution, and his procedure I, based on the determination of a temporarily maintained increment in arterial blood conductivity during constant infusion, may give erratic results because of recirculation (3). The principle has been successfully applied by Hamilton (4, 5, 6), using a non-diffusible dye instead of salt, and collecting many samples of arterial blood at about second intervals following the intravenous injection, thus permitting exclusion of the recirculated dye. Hamilton's measurements are probably as accurate as any that have been made on dog and man; his technique has not been widely employed, presumably because of the inconvenience of collecting the many arterial blood samples and determining their dye content.

It appears that a method which would permit a continuous recording of the intra-arterial concentration changes following intravenous injection, without the collection of arterial blood samples and without exposing the artery, would be advantageous; this paper reports on such a method. The objective has been to fashion a conductivity cell small enough to be inserted into an unexposed artery and to employ it as one arm of a conductivity bridge, to balance the bridge against the normal arterial blood resistance, and to follow continuously the bridge imbalance resulting from the passage of rapidly injected intravenous hypertonic sodium chloride solution; by comparing the mean height of such curve of bridge imbalance with a calibration curve obtained by titrating a blood sample against the injected solution, the mean concentration (C) of added salt during the curve of passage and the duration of such curve (T) can be obtained. Since the amount injected (A) is known, cardiac output (F) can be calculated.

Conductivity cell. Two types have been employed. The first is made by

[1] This work was aided by a grant from the Commonwealth Fund.

stretching parallel two platinum wires of about 0.2 mm. diameter about 0.2 mm. apart and coating them with an air-drying solution of some insulating plastic. Lead wires are soldered on, the assembly inserted through the nipple of a hypodermic syringe barrel and sealed in with the plastic; the lead wires emerge from the proximal end of the barrel. An 18- or 19-gauge needle is inserted into an artery and the pair of electrodes encased in their insulating cylinder inserted as a stylette, the tip of the assembly projecting just beyond the tip of the needle and lying in the blood stream, the cross sections of the wires being the electrodes of the cell. The nipple of the syringe barrel holding the cell connects with the hub of the needle in the artery.

The other type of cell is a pair of concentric hypodermic needle electrodes. The shaft of a 23-gauge needle is coated with an insulating plastic, and a lead wire soldered to the hub. The shaft of a 19-gauge needle is cut off, a lead wire soldered near its proximal end and the shaft covered with insulating plastic. This shaft is then put as a sleeve over that of the 23-gauge needle and the two firmly bound by the insulating plastic. Insulation covering the beveled ends of the needles, which are flush, is polished off, the beveled tips of the needles forming the cell electrodes. The assembly is put onto a hypodermic syringe containing a little heparin solution and bearing a barrel lock. After the needle electrodes are inserted into the artery the lumen of the inner electrode is flushed out occasionally with the heparin solution. This device has reduced the incidence of incipient clotting, which is often a source of trouble manifested by a progressive rise in base line resistance. Various types of insulating plastic have been used; it is not now possible to say which is the best;[2] most of the experiments reported were with a concentric needle cell which I prepared with General Electric's 1201 glyptol.

Source of current. It was soon found that, presumably because of the very small electrode surfaces, the conventionally employed 1000-cycle current was not suitable. Two salt solutions of known specific conductivity were prepared and the ratio of their conductivities verified by determination in a conventional type (large electrode surface) cell, using 1000-cycle current. When the ratio of their conductivities was determined using the small electrode cells described here, with 1000-cycle current, it was found to be very different from the normal and to approach normal as cycle frequency was increased, becoming normal at 70,000 cycles. This frequency was accordingly employed with these cells, the source being an electronic oscillator.

Potential recorder. No means of keeping the bridge in balance during the passage of the salt and recording continuously the compensating resistance and capacitance required to maintain such balance could be devised. Bridge imbalance is therefore permitted to take its course and is recorded on a cathode ray oscillograph. The mean height of such recorded imbalance curve can be translated into change in salt concentration by comparison with the changes in spot pattern height seen on a titration calibration curve. Bridge potentials are

[2] I wish to thank Mr. L. A. Hawkins of the General Electric Laboratories, Schenectady, N. Y., for having prepared concentric needle assemblies with formex insulation.

suitably amplified between bridge and oscilloscope; sweep speed of the oscilloscope spot is about 3.65 mm./sec. and is accurately determined at each experiment. The oscillographic record is photographed.

Calibration curve. A sample of salt solution, e.g., 0.7% sodium chloride, is put into a beaker and the conductivity cell inserted; a constant and stable reading is obtained, bridge balance being indicated by minimum pattern height (about 1.5 mm.). Equal increments of added 3% sodium chloride solution produce, after stirring, essentially equal increments in pattern height, i.e., in bridge imbalance, such imbalance being due in part to change in conductivity and in part to change in capacity. This essentially straight-line relation holds with changes in conductivity of up to about 10% and may be predicted from the following. Consider the bridge balanced, with each of the two fixed arms of resistance K, the unknown R_3 and the balancer R_4; the potential across the recorder is now zero, since $R_3 = R_4$. Change R_3 by a fraction x, producing a bridge potential imbalance, Δ v.

Then $\Delta \text{ v} = V\left[\frac{K}{K+R_3} - \frac{K}{K+(1+x)R_3}\right]$, where V is the impressed potential.

If all arms at balance are equal (which approximates our conditions) a decrease of 4% in R_3, i.e., $x = -0.04$, gives a bridge potential imbalance Δ v, which is 1.02% of V, and a decrease of 8% in R_3 gives an imbalance of 2.08% of V. Since the observed bridge imbalances produced on successive additions of hypertonic salt solution follow rather closely the above prediction, it follows that the component of bridge imbalance due to changes in capacity is approximately proportional to that due to changes in resistance, under the conditions of these determinations. In any event, we are not concerned with the relation of change in resistance to added salt but in the relation of change in spot-pattern height to added salt, and it makes no difference, for the purposes of this titration, how much of the change in pattern is due to change in capacity and how much is due to change in resistance.

The calibration curve on titrating blood with hypertonic salt offers a complication not seen when a salt solution or plasma is titrated. If, with the bridge balanced, salt solution or plasma is stirred, immediately on cessation of stirring the pattern returns to minimum height and remains stable. When blood is stirred, the pattern usually does not return promptly to its former value but shows a decelerating drift back toward the original value. This is more marked with dog than with beef blood and with heparinized than with defibrinated blood. It is probably concerned with a reorientation of the cells; the theoretical implications are probably of considerable importance, but for our present purposes the phenomenon is merely a technical nuisance. The best that has been done to date in meeting this difficulty is to wait an arbitrary number of seconds (15 to 60) after cessation of stirring, when the drift has almost or completely stopped, and to make all readings of a given titration at the same interval after cessation of stirring.

Figure 1 gives a schematic representation of the apparatus. R_1 and R_2 are 1000 ohms. Insert conductivity cell CC into an artery and balance the normal

blood resistance and capacity by adjusting R_3 and the variable condenser, giving minimum pattern height on the screen. Start the spot sweeping across the screen, inject a known amount of hypertonic salt into the vein. As the injected salt passes the electrodes, pattern deflection is recorded. The mean height of deflection is determined, and the mean concentration of added salt in blood passing the electrodes during curve is obtained from the calibration curve.

Model experiments. If a known amount of substance is dissolved in an unknown stationary volume of liquid, the volume of liquid can be determined from the resultant concentration of solute after mixing. It is obvious that movement of the liquid could not affect this relation; this principle has been shown by Hamilton and by others to afford a practical means of determining volume flow. In the present work, where concentration of added substance is not determined directly by chemical or colorimetric means but by changes in pattern height, it is necessary to show that a given increment of salt produces essentially the

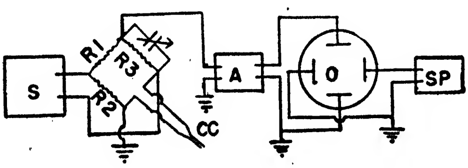

FIG.1

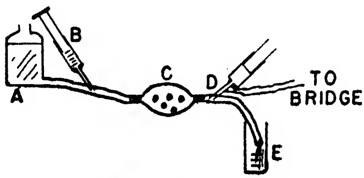

FIG.2

FIG. 1. Diagram of apparatus for recording intra-arterial conductivity changes. S source of 70,000 cycle current; R_1 and R_2, 1000 ohms; R_3, balancing resistance; CC, conduc tivity cell; A, amplifier; O, oscilloscope; SP, spreader circuit.

FIG. 2. Flow model. A, reservoir; B, injecting syringe; C, mixing bulb; D, conductivity cell; E, receiving cylinder.

same change in pattern height when added to moving fluid as when added to the same fluid in a beaker.

Experiments on running approximately 0.5% sodium chloride through the model are reported first. In figure 2 the solution is run from the container A through the mixing bulb C into the graduate cylinder E; rate of flow is varied by adjusting container height and glass tip at outlet and is measured with a stop watch, with an error of not more than 1%. With the solution running, and the bulb C being shaken, the bridge is balanced to minimum pattern height, the spot sweep started and a known amount of 3% salt solution rapidly injected at B; records of which figure 3 is typical are obtained.

A number of sets of such runs have been made; the findings of a typical set are given in table 1.

It is seen that there is complete agreement between observed rates of flow and those calculated by the present method. Completeness of mixing is proved by absence of dancing of the pattern envelope as well as by agreement between observed and calculated flows. If the mixing bulb is not shaken, the curve shows great irregularities. The reason for this is evident on inspecting the un-

shaken bulb during passage of a dye injected at B; marked streamlining of dye columns is seen, which disappears on shaking. The mixing bulb contains several stainless steel balls which are prevented from entering the bulb outlets by stainless steel coil springs inserted into the bulb stems.

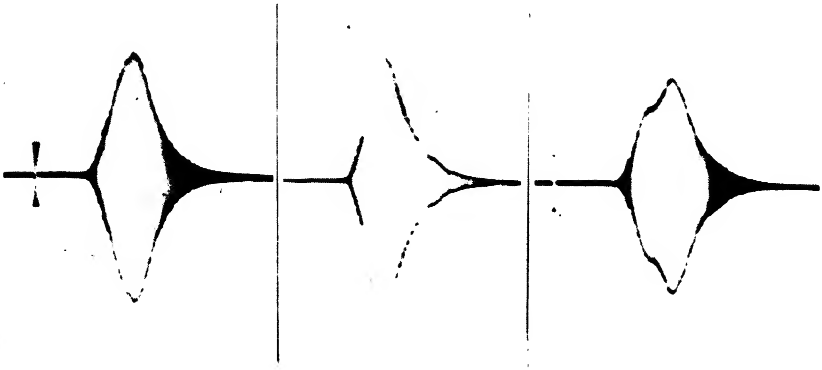

FIG. 3. Records on injecting 0.5 cc. of 3% salt at B of figure 2, while 0.45% salt is running through model. Time of injection indicated in 2 records by rectangular break in base line. Irregularity of rate of injection is shown by one asymmetrical curve; no error is introduced by this. × 6/10

TABLE 1. *Comparison of observed and conductivity method flows of salt solution*

Protocol of a series of runs made on model of figure 2. Approximately 0.45% sodium chloride solution was run through the model. Sweep speed 3.65 mm. per second, or 2.92 mm. per second on the photographed record, which is × 4/5. The calibration curve shows 1 mm. change in pattern height per 9.9 mgm. per liter of added sodium chloride. On each run 0.5 cc. of 3% salt solution, or 15 mgm., was injected rapidly at B of figure 2. Calculation of run 1. $F = \frac{60 \times 15}{16.6 \times 247} = 0.220$ L./min.

RUN	CURVE DURATION	CURVE MEAN HEIGHT	MEAN CONCENTRATION OF ADDED SALT	FLOW, CONDUCTIVITY	FLOW, OBSERVED	cond./obs.
	sec.	*mm.*	*mgm./L.*	*cc./min.*	*cc./min.*	
1	16.6	25.0	247	220	225	0.98
2	16.4	24.8	245	224	225	1.00
3	12.0	23.2	230	327	338	0.97
4	12.7	21.3	211	336	338	1.00
5	12.7	21.3	211	336	338	1.00
6	11.8	22.5	223	341	338	1.01

When defibrinated beef blood is run through the model similar results are obtained, but with some scattering. The errors with blood are somewhat greater than with salt solution because of greater difficulty of ensuring complete mixing and because the titration calibration curve is less precise than with salt solution, due to the presence of cells. The results of 3 sets of runs are shown in table 2.

On the average, the flows according to this method are higher by a small percentage than the observed, but this hardly appears to be a systematic trend. It appears that a given increment of salt concentration in flowing blood produces essentially the same change in pattern height as it does in standing, recently stirred blood.

Heart-lung experiments. Even though the flow of salt solution or blood in a model can be satisfactorily measured, there are further considerations in applying this method to the intact animal. First, there is the possibility that part of the injected salt fails to reach the left heart through temporary loss in the lungs. Evidence that about 10% of rapidly intravenously injected sodium thiocyanate escapes on its first passage through the lungs has recently been published (3), although Stewart (2) found no evidence of such escape, since cardiac output figures obtained on salt injection into the left ventricle agreed fairly with those obtained on right ventricular injection; right heart injections actually gave somewhat lower values than the left, but this finding should be taken with some reservation. Henriques (7), using sodium thiocyanate, concluded that intravenous or right heart injections were not suitable for cardiac output determinations, while left ventricular injection was; he assumed that part of the salt was lost on lung passage. He did not, however, measure the curves which can be constructed from the data of his first table on page 236 and the table on page 237. When these data are plotted and the areas of the curves measured, that of the curve from the right ventricular injection is found to be 96% of that of the left ventricular injection curve. This discrepancy is well within experimental error and can at most only indicate that a small percentage of the salt is lost on the first lung passage.

TABLE 2. *Comparison of observed and conductivity method flows of defibrinated beef blood*

Procedure as in protocol of table 1

Experiment 1			Experiment 2			Experiment 3		
Cond. flow	Observed flow	cond./obs.	Cond. flow	Observed flow	cond./obs.	Cond. flow	Observed flow	cond./obs.
cc./min.	*cc./min.*		*cc./min.*	*cc./min*		*cc./min.*	*cc./min.*	
280	244	1.15	270	273	0.99	402	426	0.95
278	243	1.14	277	277	1.00	420	424	0.99
278	249	1.12	284	281	1.01	424	423	1.00
270	253	1.07	181	182	1.00	257	235	1.09
267	245	1.09	179	185	0.97	239	234	1.02
			182	185	0.99	247	236	1.05

I have done one experiment on this point. A dog heart-lung preparation was used as described in the following paragraphs. Injections 1, 3 and 5 were made into the venous tube, 2, 4 and 6 into the left ventricle. The results are seen in table 3.

Cardiac outputs as determined on intravenous injections were 16, 14 and 6% higher than those determined on left ventricle injections, averaging 12%. The

discrepancy is interpreted as due to salt loss on lung passage and agrees well with the average discrepancy of 10% found in the heart-lung experiments described in the following paragraphs.

A further answer to this question has been sought by observations on dog heart-lung preparations. The salt solution was injected into the tube leading from the venous reservoir to the right auricle, and the electrodes put into the tube leading from the innominate artery. Left ventricular output, less coronary flow, was measured by a graduate cylinder and stopwatch. Since the conductivity method measures the entire left ventricular output, coronary flow must be added to innominate artery flow to permit a comparison. We have not determined coronary flow; values in the literature range from 5 to 15% of left ventricular output. We have assumed that left ventricular output is 1.07 times innominate artery flow in these preparations, the term 'observed cardiac output' meaning 1.07 x observed innominate flow. This factor may be slightly low; it cannot be more than 1 or 2% too high.

TABLE 3. *Comparisons of cardiac outputs as determined by intravenous and by left ventricular injections*

RUN	TIME	INJECTION SITE	FLOW	Venous injection value / Left ventricle injection value
			cc./min.	
1	2:37	vein	674	
2	2:45	l. ventricle	582	1.16
3	2:49	vein	706	
4	2:52	l. ventricle	620	1.14
Increase flow				
5	3:04	vein	828	
6	3:07	l. ventricle	782	1.06

Figure 4 gives a comparison of observed cardiac outputs so defined with those obtained by the conductivity method. These findings were obtained on a series of 10 preparations; rate of flow in a given preparation was varied by varying venous filling pressure. Figure 5 shows records of such experiments.

It is seen that the cardiac output values with the conductivity method run, on the average, about 10% higher than the observed values. This may mean *a*) that a small percentage of the injected salt escapes in the first lung passage or *b*) that the observed or true output is more than 1.07 x the innominate flow. If one assumes that coronary flow is 12 rather than 7% of left ventricular output, the observed values would be closer to the conductivity method values. We conclude that not more than 10% of intravenously injected salt is usually lost on the first lung passage in a satisfactory dog heart-lung preparation.

When pulmonary edema comes on, the discrepancy between observed and conductivity values increases, indicating increased loss of salt in the lungs; the

points in figure 4 where such edema existed are designated by E. Such edema must be marked; a few rales are quite consistent with normal cardiac output values by the salt-injection method. It seems certain that there would be no

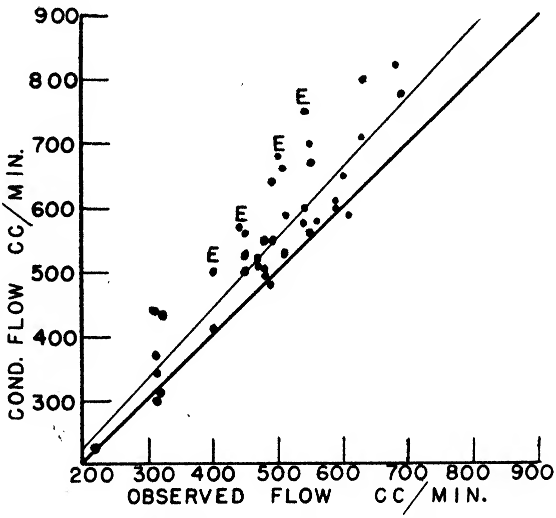

FIG. 4. Cardiac outputs of dog heart-lung preparations as determined by conductivity method, plotted against observed outputs, where observed output is taken as 1.07 × observed flow from innominate artery. Heavy line indicates position of points showing complete agreement of determined with observed outputs; lighter line shows 10% deviation. Presence of marked pulmonary edema designated by E.

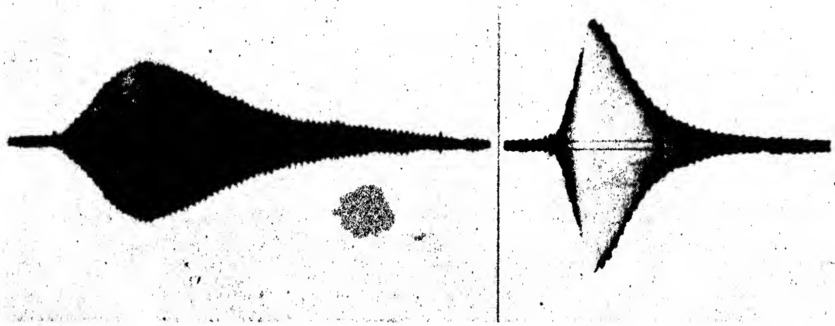

FIG. 5. Records from heart-lung preparations; time of injection not shown. × 6/10

greater salt leakage into the lungs of a normal intact animal than into those of even the best heart-lung preparation; it is probable that moderate degrees of pulmonary edema will not vitiate this method, although it is very probable that sufficiently marked pulmonary edema and congestion would cause erroneously high cardiac output values.

No discernible break in the downstroke, indicating beginning recirculation, is seen in our heart-lung records. Due to the capacity of the venous reservoir, only coronary recirculation can be present in these experiments. The records show that such amount of recirculation is too small to be recorded. With intact animals, recirculation within the period of the imbalance curve is sometimes discernible but usually not.

Intact animal experiments. Here the electrodes were inserted into a femoral artery and the 3 or 5% sodium chloride injected rapidly into a jugular vein; the dogs were anesthetized with 30–33 mgm. intravenous nembutal per kilo. Figure

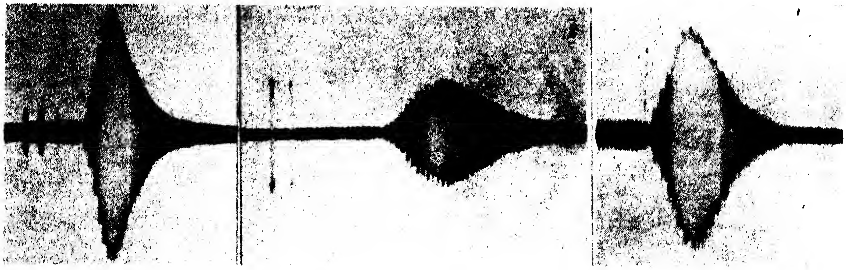

FIG. 6. Records from intact dogs. In 2 records beginning and end of injection are shown by breaks in base line. × 1/2

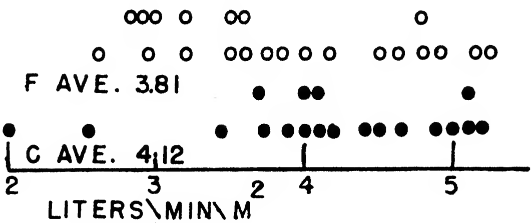

FIG. 7. Fick cardiac outputs in normal dogs as open circles, average value 3.81 L./min./M². Conductivity cardiac output values in another series of normal dogs as closed circles, average value 4.12 L./min./M².

6 shows records from intact dogs. In this series no comparisons with the Fick method were made, but Fick determinations were being made on a different series of normal identically anesthetized dogs for a different purpose. Figure 7 compares conductivity method values (closed circles) on one series of dogs with Fick values (open circles) on another series; the average of the conductivity values, 4.12 L/min/M², is 8% higher than the average of 3.81 with the Fick method.

Three difficulties not present in the model or the heart-lung experiments are encountered in the intact animal. The first is the question of recirculation during the first arterial passage, the second is the difficulty in recording sometimes seen due to improper placement of the electrodes in the artery or to the shifting of the electrode position with respect to the arterial wall, and the third is clot-

ting. Recirculation is absent in the model experiments and indiscernible in the heart-lung experiments; difficulties with electrode positioning are not encountered in the model or the heart-lung experiments, because the rubber tube serving as an artery is large enough so that the electrode surfaces remain at a safely great and constant distance from the wall; clotting is absent because defibrinated blood is used. The clotting difficulty could be obviated by heparinizing the intact animal, but it has seemed desirable to avoid this, since it is hoped that the method can be used on the human subject. It is minimized through occasional heparin flushing of the inner electrode of the concentric needle-type cell and can probably eventually be eliminated through some further technical expedient; when it is present it can be recognized by a progressive increase in base line resistance.

The second, or electrode position, difficulty makes this method unreliable, with present size cells, on small dogs, since it is not always possible to have the cell lie free in the blood stream of a small artery at a sufficient distance from the wall to ensure that the added salt has free access to the electrode surfaces and to avoid erratic changes in pattern height due, presumably, to capacity changes brought about by variations in position of electrodes with respect to the arterial wall. With dogs weighing over 15 kgm. satisfactory records can usually be obtained. Even with large dogs, and with the heart-lung preparation, pulsatile variations in pattern height are seen with each arterial pulse; i.e., even when the electrodes are safely distant from the arterial wall there are changes in resistance or in capacity, or both, with each pulse. The pattern quickly returns to its resting position between pulses, and our procedure so far has been to consider only the changes in interpulse pattern height as an index of changes in salt concentration. The mechanisms of these pulsatile changes pose problems which will require further investigation.

The question of recirculation remains to be considered. In his single rapid injection procedure Stewart recognized the arrival of the salt by the altered telephone tone; the completion of its passage was indicated by the decline in the alteration of the tone. He states (2) that "collection may be completed before a round of the circulation has been made by any appreciable part of the salt;" the evidence for this statement presumably is that the sound returned to minimum before showing a secondary rise, although there is no clear statement on this in his papers. Henriques (7) also found that all, or practically all, of intravenously injected salt had passed a given point in a dog's femoral artery before recirculation began. Hamilton and his associates, on the other hand, consistently find in both dog and man that recirculation begins before the initial arterial passage of intravenously injected dye is completed; it appears from their published curves that about 10%, sometimes up to 15%, has failed to make the first passage by the time the initial reappearance is seen. Thus, the data of experiment 11 (8), typical of their findings on dog and man, show that 8% of the area of the extrapolated 'primary' curve lies to the right of the point indicating the beginning of recirculation. In most of my intact dog records there is no discernible break in the falling limb of the curve indicating onset of recirculation;

the pattern envelope returns almost or quite to minimum height before showing a secondary rise or flattening. When a break is seen it is more often a flattening rather than an actual rise. The procedure, therefore, has been to measure on the record the area of the primary curve with a planimeter and to divide by the base line to get the mean height. When a break is seen on the downstroke, the curve is extrapolated to the base line. Comparisons of direct with semilogarithmic extrapolations have shown that no significant error is introduced by omitting semilogarithmic plotting, since that part of the area coming after the break is such a small fraction of the total. The minimum pattern envelope area (minimum pattern height x base line of curve) is subtracted from the total envelope area to get the increase due to increase in salt concentration.

TABLE 4. *Comparison of Fick and conductivity methods for cardiac output in intact dogs*

DOG	CONDUCTIVITY CARDIAC OUTPUT	FICK CARDIAC OUTPUT	Conductivity / Fick	OXYGEN CONSUMPTION	REMARKS
	L./min./M²	*L./min./M²*		*cc./min./M²*	
8	3.72	3.79	0.98	124	
12	4.03	2.87	1.47	120	
	4.43				
13	4.90	3.30	1.36	135	
	4.10				
15	4.40	3.69	1.20	133	
	4.44				
16	1.91	1.89	0.97	83.5	*Dog 16* had been hypophysectomized 13 months and thyroidectomized 8 months before this experiment.
	1.87				
	1.64				
	1.66				
	1.86				
	2.04				
17	4.05	2.77	1.60	130	
	4.60				
	4.62				
19	5.80	5.22	1.11	138	

Comparison of Fick and conductivity values. In a series of 7 dogs cardiac outputs were determined by the conductivity method, immediately followed by Fick determinations carried out as previously described (9). From 100 to 150 mgm. sodium chloride in 3 or 5% solution was injected into a jugular vein; the electrodes were in a femoral artery. The results are seen in table 4, cardiac outputs being given in liters per minute per square meter body surface.

If we accept the evidence of Hamilton and Remington (3) and of our table 3 and figure 4 that about 10% of rapidly intravenously injected salt is lost on its first lung passage, we may reduce the conductivity values of table 4 by 10%. When this is done the conductivity/Fick ratios become 0.88, 1.32, 1.22, 1.08, 0.87, 1.44 and 1.00, an average of 1.11. Since no systematic sources of error are apparent which would lead to erroneusly low values by the conductivity method, it seems probable that the cases where the ratios are less than unity

are due to erroneously high Fick values, or to inconstancy of cardiac output. It is believed that any existing cardiac output method is likely to have, for any individual determination of absolute values, an error up to about ± 15%. The conductivity/Fick ratios of 1.32 and 1.44 given above, after the tentative correction for salt lost in lung passage, almost certainly indicate a failure of the passing salt to gain free access to the electrode surfaces, due either to incipient clotting or to improper electrode position in the artery.

Circulation times. Jugular vein to femoral artery times in 13 intact normal dogs have been determined as follows (in seconds): 8, 7.5; 7, 6, 7; 5.5, 6, 7, 7; 5, 5; 7; 9; 4.9, 4.6; 7; 7, 7; 6.5, 6.5; 5, 5, 5; 8.2; and 7.9—an average of 6.7 seconds. In one dog jugular to carotid time was 5 seconds. Left ventricle to left ventricle time, i.e., from first arrival of salt to break in down-stroke, when present, has been in 12 dogs as follows: 9.5, 9.3, 8.8, 8.8; 10; 11; 7; 11, 9.5, 10; 9.5, 10, 9.5, 9; 8, 9; 9; 10; 7, 9; 8; and 9, 10—an average of 9.2 seconds. If one allows 1.5 seconds for jugular to right heart time plus left heart to femoral time, the average pulmonary circulation time (right heart to left heart) is 6.7 − 1.5, or 5.2 seconds. If this is subtracted from 9.2, the average of the left ventricle to left ventricle times, we get an average of 4.0 seconds for most rapid passage from left to right ventricle. In general, the lower times are seen with the higher cardiac outputs per M^2 for dogs of comparable size; they are lower for small than for large dogs when output per M^2 is about the same. The hypophysectomized-thyroidectomized dog, with a cardiac output of less than 1.9 L/min./M^2 and weighing 23.2 kgm., had a very long jugular to femoral time; unfortunately, the injection times were not signalled on these records but the intervals were at least 12 seconds. Left ventricle to left ventricle time cannot be determined on this dog, since there was no discernible break in the descending curve and no increase of pattern height for several seconds after completion of the curve. In many, even in the majority of instances, the beginning of recirculation is not identifiable, or is not seen until 1 or 2 seconds after the primary passage has been completed; the duration of this primary curve in normal dogs has ranged from 9 to 16, averaging 12.3 seconds. When the break is not discernible on the descending curve, the secondary rise after completion of the curve is usually so gradual that, unless the spot is completely free from drift, its beginning cannot be accurately localized. Since the average of the left ventricle to left ventricle times, 9.2, is less than that of primary arterial passage, 12.3, it appears that in most cases the first recirculation will appear shortly before the end of the primary arterial passage but that the initial amount is so low that it may be neglected. The less conspicuous evidence of recirculation in these experiments than is seen with Hamilton's dye injections is presumably because of greater salt than dye loss on first passage through systemic capillaries.

Discussion. The greatest difficulty with this method is in avoiding interference with the record through capacity or resistance changes due to movements of the arterial wall with respect to the electrodes, and in ensuring that the salt making its arterial passage gains free access to the electrode surfaces. These difficulties are greatly reduced when working with large arteries and are absent

when a tube of 7 mm. bore serves as an artery. Since man's femoral artery approximates this size, there should be less difficulty in applying the method to man than there has been with the dog; also, it may be possible to make a cell even smaller than those so far used.

It may be found advantageous to use the record in the same way that Stewart used the telephone, as a means of recognizing the end of the primary arterial passage, and collecting arterial blood as one sample throughout the period of such passage. This would avoid the necessity of multiple samples, inherent in Hamilton's technique, and the necessity of photographing and measuring the records of the present method; it would have the disadvantage of requiring another arterial puncture.

SUMMARY

1. A method for continuous recording of the impedance changes produced in a flowing stream by rapid injection of a measured amount of sodium chloride is described and is shown to afford accurate measurements of volume flow of a salt solution or of defibrinated beef blood.

2. When the method was applied to the dog heart-lung preparation the determined cardiac output values averaged about 10% higher than the observed output; this is interpreted as indicating not more than a 10% loss of salt on lung passage.

3. The method when applied to the intact dog showed fair agreement with Fick determinations, the values ranging from 13% less to 22% greater than the corresponding Fick values in satisfactory experiments.

4. Jugular to femoral time in the normal intact anesthetized dogs was 5 to 9 seconds, averaging 6.7; left ventricle to left ventricle time 7 to 11 seconds, averaging 9.2. In most cases all, or practically all, of a rapid intrajugular injection of salt passed a given point in a femoral artery before discernible recirculation occurred.

5. Circulation time was greatly lengthened and cardiac output decreased in a hypophysectomized-thyroidectomized dog.

6. Repeated determinations may be made at minimum intervals of about one minute.

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RUSSELL VIPER VENOM AS A MEANS FOR FOLLOWING THE ANTICOAGULANT ACTION OF HEPARIN

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Received for publication March 15, 1947

The purpose of this paper is to describe the use of Russell viper venom as a means for revealing both the intensity and the duration of the anticoagulant action of heparin in the dog.

METHOD. Blood was obtained by venipuncture and was decalcified by mixing with 2 mgm. of dry potassium oxalate per ml. of blood. It was promptly centrifuged and the clear plasma was diluted to 25% by adding three volumes of 0.9% NaCl. To a 0.1 ml. portion of this diluted plasma there was added 0.2 ml. of a mixture of equal parts of Russell viper venom[1], 1:10,000, and $CaCl_2$, 1%. Time was measured from the moment the venom-$CaCl_2$ mixture was added until the first flecks of fibrin appeared. For the sake of convenience this time will be designated as the R.V. time. Both the Lee-White clotting times and the prothrombin time determinations were carried out as described by Quick (1). Heparin[2] was dissolved in 0.9% NaCl and injected intravenously. More than 45 experiments on 9 different dogs were performed.

ANALYSIS OF RESULTS. It was found that the administration of heparin produced a sharp rise in the R.V. times. The intensity of the heparin action then diminished in hyperbola-like fashion, rapidly at first but more and more slowly as time progressed. This is shown in fig. 1. The plotted lines are rectangular hyperbolas which were calculated by transforming both the R.V. times and the sampling times of table 1 into logarithms and fitting the best straight lines by means of least squares. On reconverting the logarithms into antilogarithms, equations for hyperbolas were obtained.

The position and shape of the curves in fig. 1 point to the existence of a quantitative relationship between the dosage of heparin and the R.V. time curves. It will be observed that as the dose was increased both the intensity and the duration of the heparin effect also increased. This is reflected in the two parameters, k and n, of the type formula for a rectangular hyperbola. In the equation $y = kx^{-n}$, y represents the R.V. time, x the sampling time and k and n are constants for any particular curve. The following equations from the data of table 1 were obtained for the four heparin dosage levels listed below:

55 units per kgm.	$y = 127x^{-0.0716}$
110 units per kgm.	$y = 259x^{-0.2143}$
165 units per kgm.	$y = 600x^{-0.3471}$
220 units per kgm.	$y = 2145x^{-0.6524}$

[1] Supplied as 'Stypven' Brand Russell Viper Venom by Burroughs Wellcome & Co. (U.S.A.) Inc.

[2] Manufactured by Connaught Laboratories, Toronto, Canada.

Good dosage-response curves are obtained when either k or n is plotted against the dosage of heparin as is shown in fig. 2.

There are three important sources of error to be considered in these experiments. One inherent source of error lies in the hyperbolic shape of the curves discussed above. The consequence of the relatively rapid fall in the early part of the curve is that a small error in sampling time will produce a large deviation

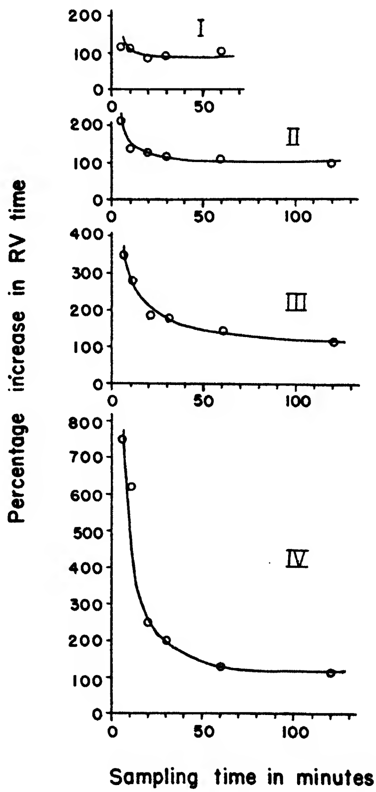

FIG. 1. The effects of graded dosages of heparin on the percentage increase in R. V. time. I, 55 units of heparin; II, 110 units of heparin; III, 165 units of heparin; and IV, 220 units of heparin.

in the R.V. time. However, the contrary is true for the latter portion of the curve. Here the rate of change of R.V. time with sampling time is small, and errors of several minutes in the sampling time are of no practical importance. A second source of error is that associated with the actual determination of the R.V. time. This is very small for the procedure yields remarkably reproducible values as is indicated by the control data of table 1. Finally, there are the errors associated with animal variation. These are considerable. Table 2 shows that

both control R.V. times and prothrombin times vary from animal to animal. Large day-to-day variations were also found in the responses of dogs to the same

TABLE 1. *Increased R.V. times produced by graded dosages of heparin*

DOSE OF HEPARIN	CONTROL R. V. TIMES	MEAN % INCREASE IN R. V. TIME AT THE INDICATED SAMPLING PERIOD[1]					
		5 min.	10 min.	20 min.	30 min.	60 min.	120 min.
units per kgm.	*sec.*						
55	28.7 29.0 28.8	119	114	87	94	107	—
110	31.2 31.6 31.0	213	139	129	119	112	98
165	26.3 26.2 26.0	353	283	190	182	147	118
220	24.3 24.0 23.8	750	620	250	202	130	117

[1] Based on triplicate determinations. The units of heparin are those assigned by the Connaught Laboratories. The same dog was used for all experiments.

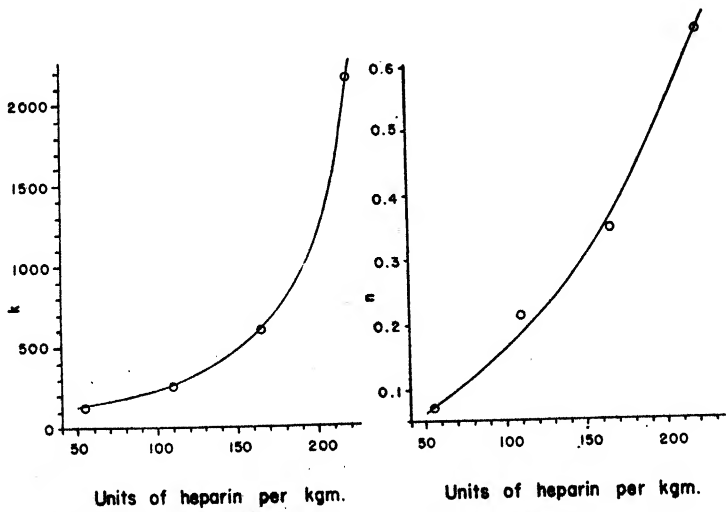

FIG. 2. The relationships of the constants k and n to the dosage of heparin.

doses of heparin. This is in accordance with the experience of Jaques (2). Therefore, it is best to design each experiment as a separate entity instead of trying to establish normal reference values or standard curves.

It is interesting to compare the results obtained with Russell viper venom with those obtained with other tests of the blood coagulating mechanism. The Lee-White times were greatly prolonged by the heparin. For example, a dose of 110 units per kgm. so altered the blood that samples taken up to 40 minutes afterward failed to clot within 8 hours. The clotting times of subsequent samples diminished rapidly, so that at 80 minutes the clotting time was 5 minutes, and at 3 hours the time was subnormal, i.e., 1.5 minutes. These results are in accordance with the well-known anticoagulant action of heparin.

In these experiments heparin had little effect on the prothrombin times as is shown in table 2, even though the comparable R.V. times were considerably increased. Such slight increases as did occur were greatest when the control prothrombin times and the control R.V. times were most nearly alike. It was found, however, that if the thromboplastin were sufficiently diluted, i.e., 1/50 of Quick's recommended concentration, that the heparin action would become

TABLE 2. *Comparative increases in R.V. times and prothrombin times after heparin*

DATE AND ANIMAL NUMBER	HEPARIN		CONTROL	OBSERVED INCREASES OVER CONTROL TIMES (IN SECONDS) AT THE DESIGNATED SAMPLING TIMES[1]				
	units per kgm.		*sec.*					
Jan. 16 *Dog D*	112	Sample		30	60	120	240	300
		R.V.	22.0	39.1	8.5	12.6	2.5	1.1
		P.T.	23.8	3.2	2.8	2.2	2.0	1.0
Jan. 15 *Dog E*	75	Sample		30	60	120	240	300
		R.V.	24.9	18.3	7.3	7.0	5.3	0.2
		P.T.	19.5	2.1	1.0	0.5	3.2	1.9
Jan. 12 *Dog C*	150	Sample		30	60	180	240	300
		R.V.	28.2	135.8	30.6	15.7	2.6	0.1
		P.T.	19.4	0.7	1.3	0.4	−0.2	0.6

[1] 'Sample' refers to the times at which the blood samples were taken in minutes after dosing with heparin. R.V. indicates R.V. times; P.T. indicates prothrombin times.

clearly evident. It may be pointed out that the times obtained under these conditions are no longer prothrombin times, since one of the fundamental principles of Quick's method is that the thromboplastin must be present in excess. It is further interesting to note that the relative behaviors of thromboplastin and of Russell viper venom as described above tend to be reversed after dicumarol treatment (3). Here, although both clotting accelerators reveal the coagulation defect induced by dicumarol, thromboplastin is more sensitive than Russell viper venom. It seems probable that the comparative results obtained with dicumarol and heparin support Edsall's (4) conclusions that the coagulating actions of thromboplastin and Russell viper venom follow independent paths.

SUMMARY

1. Russell viper venom reveals both the intensity and the duration of the anticoagulant action of heparin.

2. Graded doses of heparin produce a graded series of heparin duration curves as measured by the R.V. times.

3. R.V. times were more effective than prothrombin times in revealing the anticoagulant action of heparin in the dog.

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COMPONENTS OF THE PROTHROMBIN COMPLEX[1]

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Received for publication July 31, 1947

The concept that prothrombin is a unitary principle was generally accepted until 1943, when the writer (1) presented experimental findings that clearly indicated that this view was no longer tenable. It was shown that the diminution of prothrombin activity in stored plasma was due to the disappearance of a labile factor which was not adsorbable by aluminum hydroxide, nor was it diminished in dicumarol poisoning. This factor was designated as component A. The principle which was diminished after the administration of dicumarol and was adsorbed by aluminum hydroxide and similar agents was named component B and was considered the body of the prothrombin complex. The hypothesis that the two components were linked through calcium failed to be substantiated by later studies (2). The findings of the writer have been confirmed by Oneal and Lam (3) and by Munro *et al.* (4), but Seegers and his associates (5) have voiced their disagreement with the hypothesis and have strongly defended the unitary concept of prothrombin.

Evidence, however, has accumulated which definitely suggests that factors other than the three agents postulated in the classical theory of coagulation have a rôle in the formation of thrombin. Nolf (6) found that tricalcium phosphate removed one factor which he called thrombocyme and failed to absorb an equally essential principle which he designated thrombogene. Feissly (7), likewise, by means of fractional precipitation with sodium sulfate obtained evidence of two active fractions. Fantl and Nance (8) also have published results suggesting a new agent in plasma participating in the formation of thrombin. Zondek and Finkelstein (9) presented results indicating a new factor which they consider a co-factor of thromboplastin. Honorato (10), who has studied the labile factor, also believes it is a co-factor of thromboplastin. Very recently Ware, Guest and Seegers (11) too have obtained data on a principle other than the orthodox prothrombin which influences prothrombin activity. Most interesting has been Owren's (12) discovery of a patient with a markedly prolonged prothrombin time, which could be corrected *in vitro* by the addition of ox plasma treated with aluminum hydroxide, or, in other words, by the labile factor which the writer designated as component A, and which Owren calls factor V.

Any attempt to correlate these recent studies is beset with difficulties since each group of workers employed different experimental procedures. Furthermore, failures to recognize that fibrinogen, thromboplastin and other substances used as reagents were probably often contaminated with the new principle (13) further confuses the problem of unifying these divergent studies. Fortuitously the

[1] This research was supported by a grant from the United States Public Health Service.

writer has had available two families in which several members have a congenital hypoprothrombinemia. The fact that the defect in one family is basically different from that of the second family made it possible to obtain data which enable the writer to present a clearer concept of the factors participating in the formation of thrombin than has hitherto been possible. A clinical study of these cases has been presented. (13a)

Experimental. *Prothrombin time.* The procedure was followed exactly as recently outlined (14). The constancy of the potency of the thromboplastin was vouchsafed by the fact that it consistently yielded a prothrombin time of 11½ to 12 seconds with normal plasma and 15½ to 16 seconds on the plasma of a subject with congenital hypoprothrombinemia, who has been studied over twenty times during the past 4 years.

It should be emphasized that the experiments employed were extremely simple, and that they were limited to human plasma in which the only alteration effected was the removal of calcium by sodium oxalate, thus to meet the objection of using species with heterogeneous mixtures of plasma. Dog plasma treated with tricalcium phosphate according to the technique of Honorato (10) was used only when a high concentration of the labile factor was desired. Stored plasma was kept in open test tubes placed in a refrigerator in which the temperature was 5–8°C.

Congenital familial hypoprothrombinemia. In the first family of congenital hypoprothrombinemia studied, the mother, one son and daughter have a prothrombin time which is consistently 15½ to 16 seconds. The son, R. F., has been studied extensively and some of that data is used in this presentation.

In the second family two boys both have a prothrombin time of 19 to 20 seconds. Since both have been bleeders from birth, the defect can be considered congenital. The father and mother have normal prothrombin times. The blood of one of these boys, B. B., was used in this study, but the blood of his brother could equally as well have been used since it is indistinguishable from his.

Most of the experiments were repeated several times, except those on dicumarol plasma, which was only studied once.

Effect of storage on prothrombin activity. The experiments carried out to study the nature of the change in stored plasma are concisely summarized in table 1. From these findings one can conclude that when plasma ages, a factor diminishes which causes a prolonged prothrombin time. By mixing stored plasma with an equal volume of fresh plasma, the prothrombin time is restored to normal, which indicates that the latter contains an excess of the labile factor. The same is achieved by mixing the stored plasma and dog plasma treated with tricalcium phosphate in the ratio of 9 to 1. There is obviously a sufficient quantity of the labile factor in dog's blood to permit one-tenth of a volume to restore the amount lost in aged plasma. To avoid confusion, the principle diminished on storage is called the labile factor.

The nature of the prothrombin defect in congenital hypoprothrombinemia (family 1). From the results recorded in table 2 a number of pertinent facts stand out. Mixing fresh normal human plasma with an equal volume of R. F.'s

plasma does not bring the prothrombin time to normal but to 13½ seconds, which is the approximate expected value which should be obtained according to the author's prothrombin curve when a plasma containing 45% prothrombin is mixed with an equal volume of plasma containing 100%. The fact that fresh plasma of R. F. when mixed with stored normal plasma brings the prothrombin time to 13½ seconds (mixture III) shows that R. F.'s plasma contains as high a concentration of the labile factor as does normal plasma. On adding dog plasma treated with tricalcium phosphate to stored R. F.'s plasma (mixture V), the prothrombin

TABLE 1. *Effect of storage on prothrombin activity of oxalated human plasma*

	TYPE OF PLASMA		PROTHROMBIN TIME IN SECONDS
	Fresh normal		12
	Stored normal (6 days old)		45
	Dog $Ca_3(PO_4)_2$ treated		∞
I	Normal fresh	1 volume	12
	Normal stored	1 volume	
II	Normal fresh	9 volumes	12
	Dog $Ca_3(PO_4)_2$ treated	1 volume	
III	Normal stored	9 volumes	12
	Dog $Ca_3(PO_4)_2$ treated	1 volume	

TABLE 2. *Prothrombin defect in subject R. F. (family 1)*

	TYPE OF PLASMA		PROTHROMBIN TIME IN SECONDS
	Fresh normal		12
	Stored normal (5 days old)		32
	R. F. fresh		16
	R. F. stored (2 days old)		24
I	R. F. fresh	1 volume	13½
	Normal fresh	1 volume	
II	R. F. fresh	1 volume	16
	R. F. stored	1 volume	
III	R. F. fresh	1 volume	13½
	Normal stored	1 volume	
IV	R. F. stored	1 volume	13½
	Normal stored	1 volume	
V	R. F. stored	9 volumes	16
	Dog $Ca_3(PO_4)_2$ treated	1 volume	

is brought to its original value of 16 seconds, as is to be expected. Likewise, mixing fresh and stored plasma of R. F. yields a prothrombin time of 16 seconds (mixture IV).

It seems justifiable to conclude that R. F. lacks the conventional prothrombin which the writer has labeled component B. This factor determines the prothrombin time in normal plasma. A moderate excess of the labile factor apparently does not significantly alter this fixed value either in normal or in congenital component B deficient plasma.

The nature of the prothrombin defect due to dicumarol. It appears fairly obvious that the results obtained with dicumarol plasma (table 3) are very similar to those with R. F.'s plasma. On mixing equal volumes of R. F.'s plasma and dicumarol plasma, a prothrombin time of 16½ seconds is obtained which is fairly in accord

TABLE 3. *Prothrombin defect resulting from the action of dicumarol*

	TYPE OF PLASMA		PROTHROMBIN TIME IN SECONDS
	Fresh normal		12
	Stored normal (2 days old)		19
	Dicumarol fresh		18
	Dicumarol stored		27
I	Dicumarol fresh	1 volume	14
	Normal fresh	1 volume	
II	Dicumarol stored	1 volume	13½
	Normal fresh	1 volume	
III	R. F. fresh	1 volume	16½
	Dicumarol fresh	1 volume	
IV	Dicumarol fresh	9 volumes	16
	Dog $Ca_3(PO_4)_2$ treated	1 volume	
V	Dicumarol fresh	1 volume	14
	B. B. fresh	1 volume	

TABLE 4. *Prothrombin defect in subject B. B. (family 2)*

	TYPE OF PLASMA		PROTHROMBIN TIME IN SECONDS
	Fresh normal		12
	Stored normal (2 days old)		19
	B. B. fresh		19
	B. B. stored (2 days old)		24
I	B. B. fresh	1 volume	12
	Normal fresh	1 volume	
II	B. B. stored	1 volume	12
	Normal fresh	1 volume	
III	B. B. fresh	1 volume	11½
	Normal stored	1 volume	
IV	B. B. fresh	9 volumes	19½
	Dog $Ca_3(PO_4)_2$ treated	1 volume	
V	B. B. stored	9 volumes	19
	Dog $Ca_3(PO_4)_2$ treated	1 volume	
VI	B. B. fresh	1 volume	29
	Human fresh $Ca_3(PO_4)_2$ treated	1 volume	
VII	B. B. fresh	1 volume	29
	Physiological saline	1 volume	

with the expected value. Dicumarol causes a diminution of component B, but does not affect the labile factor.

The nature of the prothrombin defect in congenital hypoprothrombinemia (family 2). The results obtained with the plasma of subject B. B. (table 4) were

unexpected and may be devastating in their import as to the present view concerning the relation of vitamin K to prothrombin. B. B. has a prothrombin time of 19 seconds which brings him into the hemorrhagic level. On mixing his plasma with an equal volume of normal fresh plasma, the prothrombin time is restored exactly to normal. Likewise, an equal mixture of B. B.'s stored plasma and fresh normal plasma yields a prothrombin time of 12 seconds. *A priori* it appears that B. B.'s plasma lacks the labile factor, but on mixing fresh B. B. plasma with normal stored plasma, a normal prothrombin time is likewise obtained. Therefore B. B.'s plasma must contain the normal amount of the labile factor. That it is not missing in the boy's blood is further substantiated by the fact that when dog plasma treated with tricalcium phosphate is added to the fresh plasma of B. B. (mixture IV), the prothrombin time is not restored to normal and, when added to stored B. B. plasma, merely brings the prothrombin time to its original value of 19 seconds (mixture V).

The simplest explanation for these results is that B. B. lacks a factor which is neither component B nor the labile factor that disappears in stored plasma, but a distinct principle which is essential for prothrombin activity. The writer proposes that this new principle be named component A, instead of employing this designation for the labile factor as he had originally done.

The new component is adsorbed by calcium triphosphate just as is component B, for on treating fresh normal plasma with this reagent and then mixing it with an equal volume of B. B.'s plasma (mixture VI), a prothrombin time of 29 seconds is obtained, which is the same as when B. B.'s plasma is diluted with an equal volume of saline (mixture VII). Therefore, the normal plasma, which has the power to restore B. B.'s plasma to normal (mixture I), loses this ability when treated with tricalcium phosphate because this reagent adsorbs the factor which B. B.'s plasma lacks, namely component A.

Since R. F. of the first family lacks component B, and B. B. is deficient in component A, a mixture of their plasmas in the ratio of 1 to 1 should mutually correct their deficiencies. The result of this crucial experiment was as follows:

	Prothrombin time
R. F. fresh plasma 1 volume B. B. fresh plasma 1 volume	$13\frac{1}{2}$ seconds

Thus R. F. plasma with a prothrombin time of 16 seconds and B. B.'s of 19 seconds, when blended have a prothrombin time of $13\frac{1}{2}$ seconds. The plasma of B. B. has a normal concentration of component B, and R. F. has sufficient excess of component A to compensate for what B. B.'s plasma lacks. Therefore, the prothrombin time is determined by the concentration of component B in the mixture, which is approximately 70%, since R. F.'s plasma has been calculated to contain 45% of normal and B. B. has 100%.

The prothrombin defect in vitamin-K deficiency. The writer found in his records of 1938, when he studied various types of clinical vitamin-K deficiency, several observations which are significant. The first were on the plasma of a newborn baby 3 days old.

	Prothrombin time
Plasma of newborn	45 seconds
Normal human plasma	12 seconds
Plasma of newborn 1 volume; Normal plasma 1 volume	12 seconds
Plasma of newborn 1 volume; Normal plasma Al $(OH)_3$ treated 1 volume	90 seconds

The second observations were on the plasma of a jaundiced patient who later responded promptly to vitamin K.

	Prothrombin time
Jaundiced plasma	48 seconds
Normal plasma	12 seconds
Jaundiced plasma 1 volume; Normal plasma 1 volume	12½ seconds

Unfortunately these are isolated observations, and the writer has had no clinical material recently to repeat these experiments. Studies in experimentally produced vitamin-K deficiency, however, have been started and the results will be reported later. It seems justifiable to comment, however, that the observations on both the hypoprothrombinemia of the newborn and in the jaundiced patient appear not to be due to component B deficiency since the addition of normal plasma completely restores the normal prothrombin time. Since the plasma of the newborn when mixed with normal plasma treated with aluminum hydroxide prolonged the prothrombin time from 45 to 90 seconds, it seems fairly certain that avitaminotic K plasma does not lack the labile factor. One can therefore tentatively postulate that vitamin K is essential for the synthesis of component A.

Discussion. On the basis of the results presented it can be concluded that a prolonged prothrombin time by the one-stage method may be due to a diminution of any one of three different factors: component A, component B or the labile factor. Normally it appears that human plasma contains a fixed amount of component B, the conventional prothrombin of the classical theory, and that its concentration chiefly determines the prothrombin time. The second factor, component A, is present in slight excess of the actual need as shown in table 3. When this factor is diminished, it produces a hypoprothrombinemia and a bleeding diathesis indistinguishable clinically from any other type of hypoprothrombinemia. The labile factor is still an enigma. It may well be that it is a non-specific substance present in plasma to maintain a proper oxidation-reduction potential. If it can be confirmed that component A is synthesized through vitamin K, which itself has a fixed oxidation reduction potential, one is tempted to postulate that the activity of component A is dependent on the labile factor. In previous studies it was shown that the latter factor is slowly destroyed by oxidation.

According to the concept developed in this presentation the equation for the formation of thrombin is:

Prothrombin (A and B) + labile factor + thromboplastin + calcium = thrombin

The prothrombin time of the one-stage method normally measures component B, but a prolongation may be due to either component B or A deficiency. In dicumarol poisoning, component B is decreased; whereas in vitamin-K deficiency preliminary findings suggest that component A is diminished. Interestingly, a congenital defect of a fixed low level of component B in one family and of component A in another family has been shown to exist.

No attempt will be made to postulate how these various factors interact to form thrombin. The original observation of Mertz, Seegers and Smith (15) that the formation of thrombin is a stoichiometric reaction is in agreement with the writer's recent findings of prothrombin consumption in the coagulation of hemophilic blood (16). Particular caution must be exercised in using terms such as prothrombin accelerator. If the formation of thrombin is a stoichiometric reaction, it follows that its speed is governed by the law of mass action.

SUMMARY

Two families of which several members in each have congenital hypoprothrombinemia were studied. The prothrombin time in the affected members of the first family was consistently 15½ to 16 seconds. In the second family, it was 19 to 20 seconds.

A study of the hypoprothrombinemia of the first family by means of the one-stage prothrombin method showed that the blood was deficient in component B. A similar deficiency is caused by the administration of dicumarol. In the second family, the hypoprothrombinemia was due to the lack of a second factor, designated as component A. Preliminary studies suggest that it is this principle which decreases in vitamin-K deficiency.

A labile factor which disappears from stored plasma due to slow oxidation is also essential for prothrombin activity.

Present evidence indicates that in the formation of thrombin the following agents are required: prothrombin (components A and B), a labile factor, thromboplastin and calcium. There is no evidence that any of these agents are enzymes. The reaction appears to be strictly stoichiometric.

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NITROGEN CONTENT OF FEMORAL ARTERIAL AND VENOUS BLOOD IN ANESTHETIZED DOGS DENITROGENATED BY CONTINUOUS INHALATION OF 99.6 PER CENT OXYGEN[1]

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Received for publication May 10, 1947

The development of the physical and physiological concepts regarding molecular nitrogen in the animal organism has been adequately reviewed and extended (1–16). Because of the role of dissolved blood and tissue nitrogen in the genesis of intravascular bubbles, the influence of prophylactic denitrogenation in decreasing the severity of decompression sickness and aeroembolism has been repeatedly studied in both experimental animals and human subjects (3, 5–7, 10–13, 16). During an investigation of the responses of anesthetized, denitrogenated dogs to intravenously injected oxygen (17), it was observed that the decline in nitrogen content of the femoral arterial and venous blood of animals continuously inhaling oxygen was slower than that reported for dogs in the literature (5). Consequently, the femoral arterial and venous blood nitrogen content was determined in a series of deeply anesthetized dogs at successive time intervals during the continuous inhalation of 99.6% oxygen.

Experimental methods. Twenty-five normal, adult, mongrel dogs weighing from 8 to 21 kgm. were deeply anesthetized by the intravenous injection of approximately 25 mgm. of sodium pentobarbital per kgm. of body weight. Additional pentobarbital injections were administered, as required, to maintain the desired state of anesthesia. After exposure of the trachea, an L-shaped glass cannula with a diameter appropriate for the individual animal was inserted and securely tied with heavy silk ligatures. Next, the femoral artery and vein were exposed bilaterally for blood sampling, and control arterial and venous samples were simultaneously drawn from opposite sides.

Denitrogenation was achieved by the continuous administration of dry, commercial-tank, 99.6% oxygen supplied on demand, and without rebreathing, by means of an Army Air Force, low-resistance, A-16 valve connected to the tracheal cannula by a short piece of rubber tubing. The dead space in the valve and connections was not appreciably greater than that of the by-passed upper respiratory tract.

In several instances, to explore the effect of hyperventilation on rate of nitrogen elimination, a mixture of 5% carbon dioxide and 95% oxygen was employed. As no significant difference was observed between these animals and those receiving 99.6% oxygen, all the data have been combined.

Femoral arterial and venous blood samples were drawn immediately before and 10, 20, 30, 40, 60, 180, 300 and 360 minutes after uninterrupted inhalation of oxygen was begun.

[1] An abstract of this paper has appeared in *Federation Proceedings* 6: 342, 1947.

To avoid the pulmonary, passive congestion and edema which Drinker has observed in anesthetized dogs immobilized for several hours (18), the animals, which were restrained in a supine position whenever blood was drawn, were moved about every half hour from one side to the other during the experiment. Despite these efforts, however, many of the dogs exhibited, at autopsy, some pulmonary congestion.

All blood samples were collected by direct femoral arterial and venous punctures into syringes which were immediately sealed with mercury and placed in a water-ice mixture until used. The anti-coagulant was bubble-free heparin solution with which the dead space in each needle was filled before sampling. (The nitrogen content of this small volume of heparin was not great enough to influence the results). Determinations of nitrogen content were performed on each sample generally in duplicate and, at times, in triplicate by the Edwards-Scholander-Roughton technique for micro-estimation of blood nitrogen (19). Occasional checks were made by either the usual Van Slyke-Neill manometric method (20) or by a shorter modification of the latter method described by Horvath and Roughton (21). The accuracy of the determinations was equal to that achieved by Edwards *et al.* (19).

Behnke (15) has stated that there may be significant nitrogen diffusion from the ambient air into tissues of animals, particularly when open incisions have been made for blood sampling or tracheal cannulation. He cited one experiment demonstrating the difference in nitrogen eliminated through the lung in a dog exposed to 99.6% oxygen before and after closing a surgical exposure of the femoral artery and vein. In a comparable experiment the arterial and venous nitrogen content of an anesthetized dog was followed during exposure to 99.6% oxygen, as previously described. In this experiment, however, the tracheal incision was closed tightly around the cannula with sutures and then sealed with collodion; the femoral arterial punctures were made through the unbroken skin; and the venous punctures were rapidly made through a ½-inch femoral exposure which, between samplings, was held closed by means of Allis forceps on the skin and subcutaneous tissue.

Results. The complete summary of the data obtained in these experiments appears in table 1 and figures 1 and 2.

Each cited value for nitrogen, with the exception of that for 360 minutes, which is the mean of only four observations, represents the average of determinations on 10 different animals. The analysis of the data (by the method of Snedecor, 22) from 60 minutes to 360 minutes shows a significant linear regression for both arterial and venous blood ($P < 0.01$), while the deviations from regression are trivial.

It is apparent from figure 2 that in this animal, arterial and venous nitrogen contents at all time intervals did not differ significantly from those obtained in the animals with tracheal and femoral incisions closed only by cotton pads moistened with saline. This one experiment is cited with full realization that it is only one experiment. It is further discussed in the following section.

Discussion. Recently, speaking of elimination of nitrogen gas from the lungs

of anesthetized dogs receiving oxygen by tracheal cannula, Eggleton *et al.* (23) stated with that regard to the final stages of elimination, the rate does not

TABLE 1

Nitrogen content, with standard deviation of the mean, range, and coefficient of variation, of femoral arterial and venous blood in anesthetized dogs during denitrogenation by continuous inhalation of 99.6% oxygen

NUMBER OF ANIMALS	CONTINUOUS O_2 INHALATION	FEMORAL ARTERIAL BLOOD			FEMORAL VENOUS BLOOD		
		N_2 content	Range	Coef. of variation	N_2 content	Range	Coef. of variation
	min.	*vol. %* $\pm\sigma m$	*vol. %*	%	*vol. %* $\pm\sigma m$	*vol. %*	%
10	0 (controls)	1.11 ±0.023	0.98–1.17	6.7	1.12 ±0.020	0.99–1.18	5.5
10	10	0.28 ±0.019	0.13–0.34	21.1	0.45 ±0.030	0.30–0.57	21.9
10	20	0.25 ±0.015	0.16–0.31	19.0	0.39 ±0.037	0.21–0.55	29.3
10	30	0.22 ±0.014	0.14–0.27	20.1	0.34 ±0.039	0.21–0.52	35.5
10	40	0.21 ±0.018	0.12–0.33	27.2	0.31 ±0.015	0.26–0.43	15.2
10	60	0.21 ±0.012	0.15–0.29	18.4	0.27 ±0.020	0.16–0.42	24.1
10	180	0.19 ±0.014	0.15–0.25	22.4	0.24 ±0.016	0.17–0.30	21.1
10	240	0.16 ±0.016	0.09–0.24	31.6	0.20 ±0.013	0.15–0.28	20.9
10	300	0.14 ±0.011	0.06–0.16	25.1	0.19 ±0.017	0.10–0.27	28.3
4	360	0.12 ±0.020	0.07–0.18	34.2	0.15 ±0.013	0.12–0.18	17.0

Since it was not possible with the available technical aid to run the various determinations for all required venous and arterial samples from 0 to 360 minutes on any one animal, the range of the values may appear to be inconsistent. Actually, as exemplified by the composite data, the individual values were never inconsistent; both arterial and venous blood nitrogen levels steadily declined, and in no instance was an arterial value as great as the corresponding venous value.

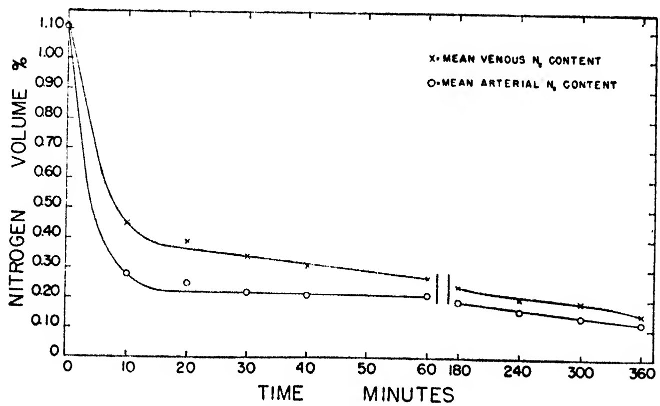

FIG. 1. Mean femoral arterial and venous blood nitrogen levels of anesthetized dogs during denitrogenation by the continuous inhalation of 99.6% oxygen.

drop to zero even after 9 hours, but falls to a low constant value of 15–30 cc/hr/-kgm., corresponding to a mean value of approximately 0.03 volume % per

minute. Yet, in one experiment in which a dog was kept in an oxygen atmosphere—to test the theory of diffusion of nitrogen into the animal from the outer air through the skin and nasopharynx—at the end of 5 hours no further nitrogen was eliminated from the lungs. However, in another similar study, these workers found that even after 7 hours the nitrogen elimination was steady at 3 cc/hr/kgm., rising to 13 cc/hr/kgm. when air replaced oxygen, implying that slightly less than 0.02 volume % of N_2 per minute could diffuse through the skin. These data suggest that individual variation is too great to allow a non-qualified statement concerning skin diffusion at this time. Certainly the experiments of Whiteley *et al.* (24) bear out this point. In the latter paper (24), in which both arterial and venous nitrogen values are in close agreement with those reported here, it is stated, as a result of experiments during which some of the animals were enclosed in an oxygen atmosphere while being denitrogenated, that the

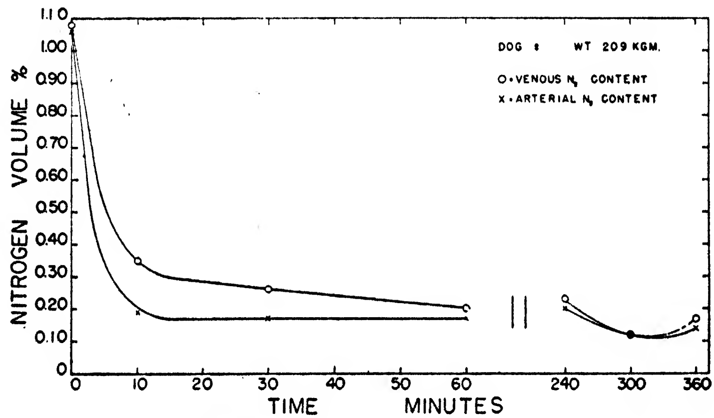

FIG. 2. The femoral arterial and venous blood nitrogen content of a dog continuously inhaling 99.6% oxygen and with both tracheal and femoral incisions tightly closed to prevent diffusion of nitrogen into the body.

prolonged maintenance of the relatively high asymptotic level of nitrogen in venous blood of resting cats cannot be primarily a consequence of diffusion of nitrogen through the skin and exposed tissues from the ambient atmosphere. It is apparent that more work must be done regarding the question of diffusion. In reference to our experiments, however, calculation of the amount of N_2 which must diffuse through the skin of a 20-kgm. dog to result in blood levels of 0.01 volume % continuously for one hour are of the order of 120 cc. of N_2 per hour—a large amount, particularly considering the hairy coverings of the animals.

In the current studies, no significantly greater denitrogenation of dogs was achieved by the use of a 5% carbon dioxide-95% oxygen mixture than by the use of 99.6% oxygen. These findings substantiate those of Henderson and Haggard (25), who calculated that with a gas of solubility as low as 0.01 (ratio of gas in one liter of blood to that in one liter of alveolar air), even a doubling of the respiration scarcely affects the rate of absorption or of elimination, while a doubling

of the circulation of the blood induces a nearly proportional increase of the rates of absorption and elimination. The reverse is true where the solubility of the gas in blood is high.

It is apparent that even continuous inhalation of 99.6% oxygen for 6 hours fails to effect complete denitrogenation of the femoral arterial and venous blood in the anesthetized dog. Although these findings do not agree with those of Shaw, Behnke *et al.* (5), who reported that nitrogen elimination is complete within 4 to 5 hours when diffusion of nitrogen into the body is prevented, it should be noted that in the experiments of Shaw, Behnke and colleagues, nitrogen values were followed not by blood analyses but by measurement of the respired gas. Moreover, Behnke and Willmon (7), on the basis of experiments with 11 human subjects surrounded by oxygen, concluded that although nitrogen is eliminated rapidly during the first 2 hours, it is eliminated slowly during successive hours, reaching an end-point within the limits of experimental error only after 9 to 12 hours.

The persistently high arterial levels of nitrogen for periods of many hours during the inhalation of pure oxygen are not in accord with current concepts of the rate of equilibration of arterial blood with alveolar air as regards dissolved gases. Nevertheless, in these experiments it has been found that the amount of nitrogen leaving the blood is not a function solely of the pN_2 in the alveoli but is a function also of the concentration of nitrogen in the venous blood.

It was empirically discovered that arterial levels can be predicted from venous levels by use of the formula $\frac{c^2}{a}$, where c represents the venous blood level of N_2 at any given moment other than equilibrium and a, the blood N_2 level at equilibrium when the animal is breathing air. Thus, the N_2 eliminated at a specific time from the venous blood is equal to $\frac{c^2}{a}$, the new arterial level being $c - \frac{c^2}{a}$. For example, in the current experiments the mean, normal, venous N_2 content a was 1.12 volumes %. At the end of 10 minutes of denitrogenation, the venous level c was 0.45 volume %. The arterial level calculated was 0.27 volume %; the mean value actually found equalled 0.28.

In the following series the arterial values calculated from the venous levels in table 1 are listed for the periods corresponding to 20–360 minutes of continuous oxygen inhalation; in parentheses are given the nitrogen levels actually found: 0.25 (0.25), 0.24 (0.22), 0.22 (0.21), 0.21 (0.21), 0.19 (0.19), 0.16 (0.16), 0.16 (0.14) and 0.13 (0.12).

The result of variations between the venous nitrogen content of different veins in the same individual is such as to make it highly unlikely that the application of the formula $c - \frac{c^2}{a}$ will consistently yield a precise estimate of arterial nitrogen in any one experiment where blood is drawn from a vein rather than from the mixed blood of the right ventricle. Presumably, the reason for the predictability of the arterial levels in the examples cited is that by virtue of the number of samples comprising the mean, the latter probably is similar to mixed, right heart venous blood, which is a composite sample of the different nitrogen values in the

venous pathways of the individual. If this is so, accurate arterial nitrogen values should be calculable in individual instances from right ventricular blood.

To test this hypothesis, determinations of N_2 levels were made on right ventricular blood at two different levels of denitrogenation, the values obtained being 0.27 and 0.29 volume %. The respective arterial levels predicted were 0.20 and 0.21 volume %. The arterial values found experimentally were 0.18 and 0.23, respectively, both well within the limits of accuracy of the microsyringe technique.

The high arterial levels of N_2 following denitrogenation and the mathematical relationship between the venous and the arterial content of N_2 suggest, on the basis of previous concepts regarding equilibration of arterial blood with alveolar gases, that, at least in dogs under our experimental conditions, there is a hitherto unsuspected factor involved in the phenomenon of N_2 exchange between blood and alveoli.

Objections may be raised that an undetected constant technical error inherent in the currently used methods of determining N_2 in the blood is the cause of our mathematical findings and that leakage, per se, is responsible for our high arterial N_2 levels during continuous denitrogenation. For the following reasons we believe such objections to be not valid.

1. Since we were able to duplicate the blank determinations of the authors, who in turn have by this method shown that they could accurately determine N_2 in solutions, the N_2 content of which was known prior to their determinations, and since, furthermore, we were consistently able to check our results by the Van Slyke-Neill and by the Horvath-Roughton modification of the Van Slyke-Neill manometric method, we feel justified in assuming that our experimental error—like that of the originators of the microsyringe technique—was certainly not greater than 0.03 volume % at all N_2 concentrations. A constant error, therefore, would not be greater than this. Yet, our arterial levels are still considerably higher than postulated by current concepts of blood-alveolar gas exchange. In fact, even doubling the error does not substantially alter this conclusion.

2. If the criticism of a technician's unsuspected error is raised, it can be pointed out that several technicians from different laboratories aided in the determinations.

3. While one must always, despite precautionary measures, admit the possibility of leakage of N_2 into the apparatus, the relative magnitude of leakage would, if it were systematic, tend to increase as the N_2 values of the blood decreased. It is quite unlikely, therefore, that leaks could have been a constant percentage of the N_2 found at increasing times of denitrogenation. In fact, under our assumption that the method at all N_2 concentrations was no more accurate than $\pm$ 0.03 volume %, the percentage accuracy decreased markedly as the N_2 concentration fell.

4. As previously indicated, the diffusion of N_2 from the ambient air surrounding the subject through the skin would have to be quite appreciable to account for an error even as low as 0.01 volume %.

5. As regards the possibility of diffusion of N_2 through the rubber connections leading from the oxygen tank to the animal, it should be noted that the system was under positive oxygen pressure. Hence, while O_2 might diffuse out, N_2 from the air could not diffuse in.

6. The physically dissolved N_2 in the pentobarbital administered during denitrogenation to maintain anesthesia was entirely negligible in these experiments. In addition, since the pentobarbital requirements among animals were variable, the anesthetic could not be the source of a constant error.

7. The presence of 0.4% N_2 in the O_2 supply was not enough to account for more than approximately 0.006 volume % of N_2, assuming negligible lipemia. Even with marked lipemia, this value would not be significantly altered.

8. Inasmuch as pulmonary congestion did occur from time to time, this may be proposed as a reason for the incomplete loss of venous N_2 from the lungs. However, not all animals showed pathology of the lung; where congestion did exist, not all had equal degrees of damage; and in no instance was there extensive pulmonary involvement. Furthermore, with the amount of congestion witnessed, normal functioning of the lungs would be expected by virtue of the presence of reserve alveolar tissue. Moreover, it would have to be a remarkable coincidence, indeed, for the pathological changes to grade themselves so precisely that a constant error would thereby be introduced and that this error would conform to values obtained before observable congestion could occur.

9. While there are data (8, 16, 26, 27) which indicate that during the process of denitrogenation the arterial N_2 may rapidly fall to levels of the order of less than 0.10 volume %, no previous experiments similar to ours have been conducted; and we cannot, therefore, compare our data with theirs, particularly regarding the applicability of the formula $\frac{c^2}{a}$, since no studies report simultaneous right and left ventricular N_2 levels.

10. The objection may be raised that the consistency of our observations regarding the calculation of arterial from right heart levels are due to chance. Statistically, the odds against this objection are 400 million to one.

11. Since resaturation would follow the desaturation pattern, it is interesting to note that Campbell and Hill (28), who measured the N_2 absorbed by the brain, liver and bone marrow of goats, came to the conclusion that only 50% saturation was attained in these tissues after 3 to 5 hours of exposure to excess pressures.

12. In their experiments on gas tensions in the blood, Riley *et al.* (29) stated that when a considerable difference in N_2 tensions exists between the (alveolar air) bubble and blood at the start of a determination, N_2 equilibration may not reach completion in 7 minutes. Serial measurements show that the bubble is still shrinking at such a time.

SUMMARY

The nitrogen content of femoral arterial and venous blood was determined at successive intervals up to 360 minutes in anesthetized dogs during denitrogena-

tion by continuous inhalation through a tracheal cannula of 99.6% oxygen, or, in several instances, by a 95% oxygen, 5% carbon dioxide mixture. Nitrogen analyses were made by the Edwards, Scholander and Roughton method, with occasional checks being made by the Van Slyke or the Horvath and Roughton manometric methods. Since the values observed with the oxygen-carbon dioxide mixture did not differ significantly from those found with 99.6% oxygen, all the data were combined.

The mean values (and standard errors) obtained at 0, 10, 20, 30, 40, 60, 180, 240, 300 and 360 minutes, respectively, for arterial blood are as follows: 1.11 (0.023), 0.28 (0.019), 0.25 (0.015), 0.22 (0.014), 0.21 (0.018), 0.21 (0.012), 0.19 (0.014), 0.16 (0.016), 0.14 (0.011) and 0.12 (0.020) volumes %. For venous blood the corresponding values are 1.12 (0.020), 0.45 (0.030), 0.39 (0.037), 0.34 (0.039), 0.31 (0.015), 0.27 (0.020), 0.24 (0.016), 0.20 (0.013), 0.19 (0.017) and 0.15 (0.013). Each cited value for nitrogen, with the exception of that for 360 minutes, which is the mean of only 4 observations, represents the average of determinations on 10 different animals. The analysis of the data (analysis of variance) from 60 to 360 minutes shows a significant linear regression for both arterial and venous blood ($P<0.01$), while the deviations from regression are trivial.

Even when allowances are made for the diffusion into the body of the animal of some nitrogen from the surrounding air, it is unlikely that complete denitrogenation can be achieved in all instances in resting dogs in less than 6 hours of continuous oxygen inhalation at atmospheric pressure.

When *c* represents the right ventricular blood level of nitrogen at any given moment other than equilibrium and *a* the blood nitrogen level at equilibrium when the animal is breathing air, the nitrogen eliminable at a specific time from the venous blood is equal to $\frac{c^2}{a}$, the new arterial level being $c - \frac{c^2}{a}$.

The authors wish gratefully to acknowledge the technical assistance of T/4 Patricia A. Gogins, W.A.C., Miss America Moreno and Mr. Thaddeus Wolczynski in the conduct of these experiments. For the analysis of variance, we are indebted to Miss Eleanor A. Donohue.

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THE MEDIATION OF FELINE ERECTION THROUGH SYMPATHETIC PATHWAYS WITH SOME REMARKS ON SEXUAL BEHAVIOR AFTER DEAFFERENTATION OF THE GENITALIA[1, 2, 3]

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Received for publication August 12, 1947

In the course of experiments designed to determine whether or not afferent impulses from the genital region play any essential rôle in the sexual aggressiveness of the male, we observed that cats deprived of the lower lumbar and all sacral segments of the cord regularly show complete erections when sexually excited. This was not an original observation, for in 1901 L. R. Müller (1) had reported that a dog from which the entire sacral cord and the greater part of the lumbar cord had been removed developed marked erections whenever it was placed with a bitch in heat, although it no longer showed reflex penile responses. This author also found that other males with cord transected at a low thoracic level never exhibited erections during displays of intense sexual excitement in the presence of an estrous female; in them, however, full erections were readily evoked by manipulation of the organ. While Müller suggested that the efferent fibers mediating erection in the absence of lumbosacral segments originate as high as the lowermost thoracic spinal segments, he reported no further investigations of this problem. This fact, together with the evidence which we had secured of the existence of a suprasacral erector outflow, led us to carry out a series of experiments the purpose of which was to determine the spinal origin and peripheral course of the fibers involved. Such a study seemed desirable in view of the possibility that this outflow may serve as a physiologically important adjunct to the erector nerves of Eckhard, which are of course part of the sacral parasympathetic outflow.

Experimental methods. *Selection and handling of the animals.* Only cats were used in this investigation. The animals were selected from males which on repeated trials regularly mounted estrous females. Such males are not always easy to obtain. Observations extending over more than 6 years have shown that a surprising number of apparently healthy mature male cats brought into the laboratory fail to become sexually active when repeatedly exposed to females in heat. When available, females spontaneously in heat were used to excite the males. Since the estrous behavior of ovariectomized females thrown into heat

[1] Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

[2] A preliminary report of some of the results reported herewith appeared in abstract form in this Journal **119**: 392, 1937.

[3] This work was begun when one of us (W. S. R.) was a member of the Department of Physiology, University of Maryland School of Medicine.

by administration of an estrogenic substance is identical with that of spontaneous estrus (2), it seemed unobjectionable and was certainly more convenient to employ such animals. Routinely we induced estrus by intramuscular injections of estradiol proprionate in oil (Progynon B).

Whenever a male was tested with an estrous female the latter was placed in the cage of the former or in an area to which the male had become accustomed. It was early found that any strange physical environment exercises a markedly dampening effect on the sexual proclivities of almost all male cats. The behavior of the pair was noted and the sequence of events in the sexual pattern of the male was carefully recorded. The state of turgidity of the penis was determined by palpation. After a little experience it was easy to state with confidence that an erection, if not full, was one-fourth, one-half or three-fourths complete.

Surgical procedures and postoperative care. All operations were carried out under deep pentobarbital sodium anesthesia (42 mgm. per kgm. of body weight, intraperitoneally). Spinal segments were extirpated after exposure of the cord in the usual way. The various segments were identified by locating the 7th lumbar nerve roots which are usually the largest in this region. The cord and dura were cut through below the 3rd sacral segment (S3) and then at a selected higher level after which all intervening roots were sectioned extradurally. The length of cord, encased in dura with cut roots protruding, was removed from the spinal canal and at once fixed in formalin for future reference. After the animal had died or was sacrificed, the extent of the extirpation was verified by removing the entire remaining cord with roots attached.

In certain instances, as a second procedure, the cord was sectioned some distance above the region of removal. A number of animals were subjected to one or more of the following operations: *a*) bilateral removal of the entire sympathetic chains below the diaphragm; *b*) bilateral removal of the sacral portions of the sympathetic chains and *c*) resection of the proximal portions of the hypogastric nerves with or without extirpation of the inferior mesenteric ganglia. Each of these was carried out through a midline abdominal incision.

In all operations, especially those involving cord extirpations, considerable care was taken in closing the wound. Muscle layers and subcutaneous tissues were brought together by discontinuous sutures. After firmly approximating the skin edges by buried sutures the closure was completed by a cuticular suture of very fine silk.

After operation the animals were kept in large individual cages. Daily observations were made and records kept of their food intake and defecations. After every cord operation the bladder was emptied at least once daily by manual pressure. Although a certain amount of dribbling occurred, this prevented over-distention of the bladder which tends to interfere with the blood supply and may result in infection and erosion of the mucosa. In confirmation of Langworthy and his collaborators (3) we observed that the bladders of animals which survived removal of the sacral cord for any considerable time were hypertrophied, the mucosa being thrown into large folds.

Results. *Sexual behavior of the normal male cat.* Sexually active male cats

will copulate daily during the fall, winter and spring months. Some very active males will copulate as many as five times during a period of one or two hours, but the most energetic males of our series showed less stamina as regards frequency of the sexual act than did the fully estrous females. The animals appear to pay no attention to the observer; it is our impression that once a male cat mounts a female cat little short of a gross mechanical disturbance will interfere with the continuance of his efforts.

When the sexually active male cat is exposed to a female cat in heat, he usually watches her intently for a few moments. Then he utters a chirping cry and seizing the nape of her neck with his teeth quickly mounts her. The male now executes stepping or treading movement with his hindlegs. As excitement increases this motor activity is succeeded by copulatory movements of the pelvis. Dexterous males often intromit at the first attempt. When the male is less skillful, or if the female requires more genital stimulation before her cooperation is obtained, intromission may be delayed. In the case of most males insertion of the penis for one or two seconds appears to result in ejaculation. On intromission the female emits a loud cry and quickly dismounts the male by moving forward or by turning to strike him after which she engages in the stereotyped post-copulatory behavior characteristic of the female cat (2). Only rarely does a male possess sufficient vigor to retain his grasp of the female and immediately to repeat the act of coition. Usually he releases her and after licking his penis watches her antics. A second copulation may take place within a few minutes or it may be delayed for half an hour or longer. After several intromissions male cats become unusually pugnacious and run about striking and spitting at other males or even at the female which had just been the object of their attentions.

Erection in the normal cat. The male cat, unlike the male of many species, rarely if ever shows an erection in the mere presence of an estrous female. None of the 272 complete and 3 incomplete erections which we have observed in 25 normal cats developed until after the male had seized the female by the neck and mounted her. Under these circumstances a full erection usually appeared within a few seconds, but occasionally it was delayed for 1 or 2 minutes. Its development was always rapid. Once the erection became complete it was maintained until the male dismounted. Detumescence was also usually rapid, but if the animal repeatedly licked his penis the erection was maintained for some time. Intromission or ejaculation appeared to exert little or no influence upon the rate of detumescence. In the normal male cat it proved impossible, except immediately after the subsidence of an erection accompanying sexual activity, to evoke any degree of penile turgidity by manipulation of the organ. Such a procedure almost always induced vigorous signs of anger, and it may be that a vasoconstrictor discharge associated with this form of excitement precluded reflex penile vasodilatation.

Sexual responses of male cats after removal of lumbosacral segments of the spinal cord. Removal of the lower segments of the spinal cord was carried out as the first operative procedure in 21 cats (in all animals of tables 1, 2 and 3 except *cat 3*). Each of these males had demonstrated marked sexual activity before opera-

tion. In some the extirpation extended as high as L5 or even L4 and in every instance it included the three sacral segments. The cats were tested by exposing them repeatedly to estrous females over postoperative periods which in most cases were of several weeks duration. Of the 154 tests carried out on these animals 129 resulted in full erections, 19 in incomplete erections and 6 in no penile response. Every failure to develop a complete erection occurred during the first few days after cord removal; on later tests full erections were always obtained.

The sexual behavior of these cats with cord removals differed somewhat from that shown by normal animals. When any one of these males was exposed to an estrous female he immediately seized the nape of her neck with his teeth and mounted as best he could, then executed limited but distinctive copulatory movements the extent of which depended on the amount of axial musculature still innervated. All these animals would remain in position for at least several min-

TABLE 1. *Animals with only ablations of lower segments of spinal cord*

CAT NO.	SEGMENTS REMOVED (INCL.)	POSTOPERATIVE OBSERVATION PERIOD	TIMES TESTED	ERECTIONS			REMARKS
				Full	Incomplete	None	
		days					
4	S1–S3	3*	3	3	0	0	*Died after a 2nd operation on 4th day
22	L7–S3	14	4	3	1*	0	*About 90 per cent full
7	L6–S3	30	8	8	0	0	
9	L6–S3	45	17	16	1	0	
10	L6–S3	27	9	9	0	0	
16	L6–S3	10	7	6	2*	0	*First 2 tests
26	L6–S3	41	11	11	0	0	
21	L6–S3	12	7	5	1*	1†	*Nearly complete; †on 1st postoperative day

utes. We have often noted that even after 30 minutes of uninterrupted activity such males could be separated from the female only by force. Repeated tests showed no diminution in their sexual aggressiveness, although the deafferentation of the genital region and the paralysis of the hindlegs prevented intromission. On many occasions there could be no doubt that the erection developed as rapidly as in normal animals. Also, as in the case of normal animals, detumescence occurred rapidly after dismounting. Reflex erection could not be produced by manipulating the penis, abdominal wall or any part of the body. In every case there was complete anesthesia of the genital area. No reactions of any kind could be elicited by strong nociceptive stimulation of penis, scrotum, perineum, anal region or tail. The further extent of the anesthetic area of course depended on the magnitude of the cord extirpation.

Effects of transection of spinal cord at upper lumbar or lower thoracic levels on the capacity to develop erections after exclusion of the sacral parasympathetic outflow. Six cats which had shown full erections after removal of the sacral and two or

more of the lumbar segments were subjected to cord transections at various upper lumbar or lower thoracic levels. The results appear in table 2. When the transection was made between L2 and L3 (*cat 12*) full erections were not seen in any of the 14 tests made during a period of 17 days. Eleven of the tests, however, gave incomplete responses, three of which approximated full erections. *Cat 17*, in which the cord was cut between L1 and L2, developed only one erection,

TABLE 2. *Effects of transections of spinal cord at an upper lumbar or lower thoracic level on erections evocable after exclusion of sacral parasympathetic outflow*

CAT NO.	SURGICAL PROCEDURES		POSTOPERATIVE OBSERVATION PERIOD	TIMES TESTED	ERECTIONS			REMARKS
	Date	Segments removed Transections Sympathectomies			Full	Incomplete	None	
			days					
12	5-20-37	L6-S3, inclusive	17	5	5	0	0	
	6- 7-37	Sacral chains out	13	7	7	0	0	
	6-21-37	Cord cut between L2 and L3	17	14	0	11*	3	*On 3 occasions nearly complete
17	6-10-38	L6-S3, inclusive	11	6	5	0	1*	*On 1st test, 2nd day
	6-21-38	Cord cut between L1 and L2	30	17	0	1	16	Great excitement in all tests
18	2-17-39	L5-S3, inclusive	15	5	5	0	0	
	3- 4-39	Cord cut between T13 and L1	12	2	0	0	2	Tested on 5th and 10th days; sustained activity, great excitement
19	4- 4-39	L4-S3, inclusive	92	11	6	1	4*	*First 4 tests
	7- 6-39	Cord cut between T13 and L1	58	14	0	0	14	Great excitement and sustained activity on each test
20	6-19-39	L6-S3, inclusive	18	7	7	0	0	
	7- 8-39	Cord cut between T13 and L1	14	8	0	0	8	Great excitement and sustained activity on each test
13	6-14-37	L5-S3, inclusive	15	4	2	2	0	
	6-30-37	Sacral chains out	8	7	6	1	0	
	7- 9-37	Cord cut between T11 and T12	19	13	0	0	13	Great excitement and sustained activity on each test

an incomplete one, although the animal was sexually aggressive and showed signs of considerable excitement on each of 17 trials. The three animals (*cats 18*, *19*, *20*) with cord sections between T13 and L1 and *cat 13*, in which the cord was severed between T11 and T12, were tested repeatedly but, despite the exhibition of great sexual excitement, never showed any signs of an erectile response.

Effects of removal of various parts of the sympathetic innervation combined with

Table 3. *Effects of removals of various parts of the sympathetic innervation combined with ablation of lower spinal segments*

CAT NO.	SURGICAL PROCEDURES: Date	SURGICAL PROCEDURES: Segments removed Sympathectomies	POSTOPERATIVE OBSERVATION PERIOD	TIMES TESTED	ERECTIONS: Full	ERECTIONS: Incomplete	ERECTIONS: None	REMARKS
			days					
1	5- 9-35	L7-S3, inclusive	5	5	5	0	0	
	5-16-35	Abdominal chains and i.m.g. out	131	20	0	0	20	Tested on 18 of 1st 28 days and on 115th and 131st days
2	2-14-36	L7-S3, inclusive	34	7	7	0	0	
	3-19-36	Abdominal chains	26	8	0	0	8	Tested on days 1, 2, 4, 13, 21, 22, 25, 26
3	5- 9-36	Abdominal chains and i.m.g. out	31	10	10	0	0	
	6-10-36	S1-S3, inclusive	22	11	0	0	11	9-11 min. continuous activity at each test
5	9-18-36	L6-S3, inclusive	19	9	4	5*	0	*All greater than ¾ complete
	10- 7-36	I.m.g. and hypogastric nerves	180	24	0	0	24	5-10 min. continuous activity at each test; tested on 180th day
8	1-19-37	L5-S3, inclusive	23	5	5	0	0	
	2-11-37	I.m.g. and hypogastric nerves	140	39	0	0	39	
14	6-25-37	L5-S3, inclusive	21	3	3	0	0	
	7-17-37	Sacral chains	11	4	4	0	0	
6	12- 1-36	L6-S3, inclusive	33	9	7	2*	0	*Both nearly complete
	1- 4-37	Sacral chains	35	11	11	0	0	
	2- 8-37	I.m.g. out	42	14	0	0	14	7-9 min. continuous activity at each test
15	5-21-38	L6-S3, inclusive	27	12	8	4*	0	*On first 4 tests
	6-17-38	Hypogastrics	13	10	0	5	5	
	7- 1-38	I.m.g. out	14	9	2	5	2†	†On 1st and 2nd days
	7-16-38	Lower lumbar chains sectioned	6	2	0	0	2	

In third column i.m.g. indicates inferior mesenteric ganglia.

ablation of lower spinal segments. (*a*) Complete sympathetic denervation of the genital organs before or after extirpation of lower spinal segments was carried out

in *cats 1, 2* and *3* (table 3). *Cat 1* was sexually aggressive and showed full erections on each of 5 daily tests made after extirpation of cord segments L7–S3, inclusive. Following removal of both abdominal chains from diaphragm to pelvic rim and excision of the inferior mesenteric ganglia no trace of an erection was observed, although he seized and did his best to mount estrous females on each of the 20 tests carried out during 131 days of survival. Similarly, in *cat 2* extirpation of the entire cord below L6 in no way diminished the capacity to develop erections, but after bilateral removal of the abdominal sympathetic chains no erectile response occurred during 8 wholly satisfactory tests made over a period of 26 days. *Cat 3* was subjected to essentially the same procedures as *cat 1* but in reverse order. He showed a full erection in each of the 10 tests performed after removal of the abdominal chains and the inferior mesenteric ganglia. The three sacral spinal segments were then extirpated. No erection occurred during any of the 11 tests made during the following 22 days. It thus became quite clear that abdominal sympathetic pathways carry the impulses which effect penile erection in the absence of the sacral cord. The following procedures were carried out to determine more precisely the course of these impulses.

(*b*) In 4 animals (*cats 12* and *13*, table 2; *cats 14* and *6*, table 3) extirpation of the lower spinal segments was followed by bilateral removal of the sacral portions of the sympathetic chains. These males were tested repeatedly during postoperative periods which varied from 8 to 35 days and on every occasion but one (one of the 8 tests of *cat 13*) quickly developed a full erection. It appears certain enough that in these cats the vasodilator discharge reached the penis by a route other than the sacral sympathetics.

(*c*) In 3 animals (*cats 5, 8* and *6*, table 3) removal of the inferior mesenteric ganglia—with resection of the proximal portions of the hypogastric nerves in *cats 5* and *8*—completely abolished the capacity to develop erections which had fully survived ablation of the spinal origin of the sacral parasympathetic outflow and, in the case of *cat 6*, removal of the sacral chains as well. It is to be emphasized that each of these animals was subjected to rigorous testing. Following inferior mesenteric ganglionectomy *cats 5* and *8* remained sexually aggressive and were exposed many times to estrous females over postoperative periods of 180 and 140 days, respectively. *Cat 6*, which had undergone a long study before ganglionectomy, was tested 14 times during 42 days; despite continuous, prolonged and vigorous excitement in each test he never once showed any trace of penile erection.

(*d*) The results presented above indicate that the sympathetic vasodilator discharge passes to the penis by way of the hypogastric nerves. That the sacral sympathetic chains may be involved in some cats is suggested by the results obtained in the case of *cat 15* (table 3). After full recovery from removal of the three sacral and the two lowermost lumbar segments of the cord this animal showed complete erections whenever he seized an estrous female. With the intention of interrupting the effective pathway we sectioned the hypogastric nerves just distal to the inferior mesenteric ganglia. In 5 of 10 tests carried out

during the ensuing 13 days an incomplete erection occurred. Thinking that we had probably missed some strands of the hypogastrics we then removed the inferior mesenteric ganglia. Following this third operation the cat was tested 9 times in 14 days. On the first 2 tests no erection was seen, but 7 later tests brought forth 5 incomplete and 2 complete erections. The lower lumbar sympathetic chains were then sectioned after which no erection occurred in the 2 tests carried out during the subsequent 6 days.

The effect of abdominal sympathectomy alone. In view of the fact that when the sacral parasympathetic outflow, the nervi erigentes, is eliminated a sympathetic innervation of the penis is capable of mediating erection during sexual excitement, it seemed desirable to examine the effects of sympathetic devervation of the genitalia in animals which were otherwise wholly intact.

In 2 cats, *11* and *25*, observations were made after removal of the entire extent of the sympathetic chains below the diaphragm. The same operation, combined with inferior mesenteric ganglionectomy, was performed in 3 other animals (*cat 3*, table 3; *cats 23* and *24*). The genital responses of these 5 animals were studied over periods which varied from 10 to 31 days. A total of 49 tests were made in each of which the males exhibited the usual aggressive behavior. In 48 of these bouts full erections developed and intromission took place. The single failure occurred in the first postoperative test on *cat 23*. Although the animals displayed, without outward modification, the full sexual behavior pattern of the normal male cat, the operations rendered them incapable of ejaculation, as was shown by failure to find any spermatozoa in vaginal smears made immediately after copulation.

Discussion. The method of studying the sexual behavior of animals which have long survived one or more surgical procedures possesses the merit that the responses observed are evoked under physiologically normal conditions. When a positive response—in this investigation erection of the penis as an accompaniment of sexual excitement—is obtained, the result is convincing. We are not insensible of the fact that our negative findings do not have quite the same standing. However, our negative results were obtained on long surviving healthy cats many of which had been subjected to surgical procedures no more extensive than those practiced on animals which gave fully positive results. We have no doubt that almost all of our negative results were due to the interruption of specific nervous pathways. When, for example, after a bout lasting 30 minutes, a mounted male cat, on being forcibly separated from the female, spits and strikes at the observer and crying plaintively makes repeated attempts to gain access to the female, it seems reasonable to conclude that his penis had remained flaccid for want of a neural connection with the site of central excitation.

Delimitation of the suprasacral vasodilator outflow to the penis was attempted by determining the effects of cord transection at different levels in a series of animals which had shown full erections after extirpation of a length of cord consisting of the three sacral and two or more of the lower lumbar segments (table 2). When the section was made above the first lumbar segment, the capacity to develop any erectile response in the course of sexual excitement was abolished.

When the transection was at a somewhat lower level, namely between L1 and L2, a slight erection developed. Only incomplete erections were obtained after transection between L2 and L3, but they were greater in number and extent. These results were supplemented by finding that full erections regularly developed in animals from which all spinal segments below L4 had been removed (tables 2 and 3). This indicates that an important part of the outflow issues from L3 and L4. There is a suggestion in our results that the capacity to show full erections was somewhat affected after an extirpation which included L4, whereas removals which just spared this segment (and its roots) had no such effect. Thus it appears that the outflow under consideration is composed chiefly of fibers which originate from the 2nd, 3rd and 4th lumbar segments. The 1st lumbar segment may contribute a few fibers. This conclusion is in quite close accord with the facts established by Langley and Anderson (4) concerning the origin in the cat of the lumbar outflow of the sympathetic innervation of the generative organs, both external and internal. They found that the lowermost segment involved is the 4th lumbar when there is an anterior arrangement of nerves, the 5th, when the arrangement is posterior.

The suprasacral vasodilator pathway to the penis runs through sympathetic channels. Thus cats in which extirpation of the spinal origin of the nervi erigentes is followed by bilateral removal of the abdominal sympathetic chains do not develop erections when they seize and mount estrous females. It seems clear that in most instances the dilator fibers do not reach the pelvis via the sacral sympathetic chains, for bilateral removal of these in 4 cats in which the lower spinal segments had been extirpated previously did not prevent or retard the appearance of full erections. On the other hand, no trace of an erection was obtained in 3 animals in which inferior mesenteric ganglionectomy was combined with removal of the lower spinal segments. It appears therefore that the usual pathway from lumbar segments to penis involves the lumbar portion of the abdominal sympathetic chains, the inferior splanchnic nerves, the inferior mesenteric ganglia and the hypogastric nerves. We have encountered only one exceptional case (*cat 15*). In this instance the erectile response shown after extirpation of lumbosacral segments was not abolished by removal of the inferior mesenteric ganglia, but disappeared after section of the lower lumbar sympathetic chains.

Our finding that fibers mediating erection are present in the hypogastric nerves is in accord with the results of several early investigators of the erectile mechanism. Both Eckhard and Budge (quoted by Langley and Anderson, 4) produced erection in the rabbit by stimulation of the hypogastrics. This was later confirmed by François-Franck (5) in experiments on dogs. More recently Bacq (6) has reported that hypogastric stimulation in dogs usually causes a slight increase in the volume of the glans penis and that eserine greatly increases and atropine abolishes the response. On the other hand, Langley and Anderson (4) in the course of their many stimulations of the hypogastrics in rabbits, cats and dogs never saw erection or dilatation of blood vessels. We also have been unable to obtain any degree of erection on peripheral stimulation of the hypogastrics in

the anesthetized cat or dog or in the unanesthetized decerebrate cat. Doubtless one factor contributing to these failures is the masking effect produced by concurrent stimulation of vasoconstrictor fibers to the penis which are known to be present in the hypogastrics (4, 7). During sexual excitement the vasodilator component is obviously selectively activated.

In agreement with Bacq's (8) experiments on rodents, we find that male cats from which the abdominal sympathetic chains have been removed do not expel semen during coitus. On the basis of the illuminating experiments of Semans and Langworthy (7) this defect is due to failure of the mechanism whereby seminal fluid is transported from the seminal vesicles into the posterior urethra. This process of emission depends on contractions of the vasa deferentia and seminal vesicles which are brought about, as has long been known, by impulses in fibers of the hypogastric nerves. Semans and Langworthy have demonstrated that in the cat ejaculation, the actual expelling of semen through the meatus, is a separate act which depends on contractions of striated muscles under control of the internal pudendal nerve. Aside from failure to inseminate females, the sexual behavior of our abdominally sympathectomized males was entirely normal. They gave no indication of the 'incomplete copulation' observed by Bacq (8) in male rabbits deprived of their abdominal chains, a deficiency which he attributed to their inability to ejaculate.

The unattenuated sexual excitement exhibited by male cats with lumbosacral segments of the cord and abdominal sympathetic chains removed shows conclusively that afferent impulses from the pelvic viscera, the external genitalia and a wide area of skin surrounding the external genitalia are not necessary for the initiation or the maintenance of sexual activity. Over periods of many months such animals will indulge repeatedly in prolonged attempts to copulate without showing any diminution in their sexual aggressiveness. In this respect the male is like the female. One of us (9, 2) has previously reported that in the female cat complete denervation of the genitalia and of all possible erogenous zones surrounding the external genitalia in no way diminishes the conspicuous 'courtship' activities of feline estrus.

SUMMARY

1. Male cats deprived of the spinal origin of the nervi erigentes regularly develop full erections when they seize and attempt to mount estrous females.

2. The suprasacral vasodilator (erector) outflow is composed of fibers which originate chiefly from the 2nd, 3rd and 4th lumbar segments of the spinal cord. The pathway runs through the lumbar portion of the sympathetic chains and in most cases reaches the pelvis by way of the inferior mesenteric ganglia and hypogastric nerves.

3. Exclusion of afferent impulses from the genitalia and indeed from the whole pelvic region and tail, including all skin areas surrounding the external genitalia, does not influence the sexual aggressiveness of male cats.

4. Abdominal sympathectomy abolishes ejaculation (emission), but in no other way alters the sexual behavior of male cats.

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EFFECT OF THYMECTOMY, HYPERTHYROIDISM AND HYPOTHYROIDISM ON NEUROMUSCULAR ATROPHY AND REGENERATION[1]

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Received for publication August 15, 1947

That certain endocrine dysfunctions are accompanied by muscular pathology has long been known(1). Hyperthyroidism is often accompanied by creatinuria, atrophy, fine tremors, myasthenia and lesions in the skeletal muscles (2). Remission of the creatinuria and asthenia has been reported to occur following thyroidectomy (2, 3). The possibility that muscular weakness accompanying myasthenia gravis may be due to the liberation of a curare-like substance by the thymus gland has been considered (4). In some cases thymectomy has been followed by an amelioration of the symptoms of this disease (5). The object of the present study was to determine the effects of thymus deprivation, hyperthyroidism and hypothyroidism on the course of neuromuscular degeneration and regeneration in the albino rat.

Experimental methods. Thirteen albino rats, 2 to 3 months old, were thymectomized according to the method of Einhorn (6). Fifty-two days were allowed for the development of any states of thymic deficiency, after which time these animals and a control group of 15 rats of the same age and body weight were subjected to a crushing of the left tibial nerve at its juncture with the peroneal nerve. The unoperated contralateral limb was used as a control. Thirty days after crushing the nerve, measurements were made under light ether anesthesia of the strength and weight of control and regenerating muscles. This was done by measuring the maximal isometric tension developed by the muscles upon direct and indirect stimulation. For this purpose the tendon of Achilles was cut and attached to a Blix-type torsion rod and a portion of the femur was exposed and fixed in a rigid clamp. The intact muscle was stimulated by volleys of slightly super-maximal induction shocks applied through two needles which pierced it, one at the tendon and the other near its origin. Adjustable silver electrodes were placed in contact with the tibial nerve which delivered volleys of condenser discharges. The frequency and strength of the stimuli were such as were found to be adequate to evoke maximal isometric tetanus tension responses in the muscle. The extent of muscle shortening was measured from optical records, and the strength was considered to be the maximal tension which developed in response to either direct stimulation of the muscle or its motor nerve. At the conclusion of the strength measurements the gastrocnemii were dissected out and weighed.

In the thyroid studies 128 albino rats, 2 to 4 months of age, were divided into 3 groups and matched as to age, sex and body weight. Hypothyroidism was induced in one group by feeding ad libitum a diet of powdered Purina Laboratory

[1] Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

Chow to which had been added 2 grams of thiouracil per kilogram of food. This diet has been shown by Barker (7) to produce a state of hypothyroidism with a decrease of about 20% in the metabolic rate. Animals in this group were placed on the diet 2 weeks prior to nerve crushing. Hyperthyroidism was induced in a second group of 43 animals by subcutaneous injection of 1.5 mgm. for females and 2.0 mgm. for males of thyroxin twice a week. Thyroxin injections were begun at the time of the denervation operation. A third group of operated untreated animals served as controls. All animals were subjected to a crushing of the left tibial nerve. Measurements of muscle strength and weight were made as described above at 10–14, 21, 28 and 42 days after denervation. Experiments were performed to determine the time after denervation at which the earliest signs of functional reinnervation appeared. This was done by determining the earliest time at which stimulation of the regenerating motor nerve would cause the slightest detectable movement of the gastrocnemius muscle.

TABLE 1. *A summary of average values and standard errors for relative weight and strength of gastrocnemii at various times after denervation. Values are expressed as per cent of those in non-denervated contralateral controls*

TIME AFTER DENERVATION	RELATIVE WEIGHTS OF MUSCLE			RELATIVE STRENGTH IN RESPONSE TO NERVE STIMULATION			RELATIVE STRENGTH IN RESPONSE TO DIRECT STIMULATION		
	Thyroxin	Control	Thiouracil	Thyroxin	Control	Thiouracil	Thyroxin	Control	Thiouracil
days	%	%	%	%	%	%	%	%	%
10-14	60.7±1.6	63.9±1.4	75.0±3.1	0	0	0	27.6±2.8	30.3±2.8	39.2±3.2
21	54.2±1.4	64.1±2.2	74.9±2.6	26.1±2.9	20.2±2.0	19.5±1.8	36.4±2.6	40.9±2.3	46.0±2.4
28	68.8±1.9	65.2±1.5	69.4±1.8	44.2±3.1	40.6±3.1	35.6±2.9	54.1±1.7	50.4±2.7	50.6±1.3
42	83.2±3.1	76.5±1.2	73.4±3.9	72.3±3.9	61.7±1.8	53.4±2.6	75.1±3.7	65.9±2.1	62.1±2.7

Thymus experiments

	Thymectomized	Control	Thymectomized	Control	Thymectomized	Control
30	63.9±1.7	66.7±2.7	48.6±3.5	48.7±3.7	62.5±4.3	61.2±5.1

RESULTS. No significant difference in weight and strength between regenerating muscles of thymectomized and control animals (table 1) was found. There was no significant difference between control and thymectomized animals in the response to nerve stimulation and direct stimulation and in the amount of functional reinnervation as measured by the ratio of tension responses to direct and indirect activation.

The onset of functional reinnervation appeared earlier in thyroxin-treated than in control animals, but there was no difference in the time of onset of functional reinnervation between control and thiouracil-treated animals. Additional evidence for this effect of thyroxin was afforded (table 2) by the finding of a higher ratio of tension from nerve stimulation to that from direct muscle activation at 21 days after denervation in thyroxin-treated animals than in either the control or thiouracil-treated groups.

The data presented in figure 1 and tables 1 and 2 show that thyroxin adminis-

TABLE 2. *A summary of average values and standard errors for the ratios of the isometric tension response from nerve stimulation to direct stimulation and the weight of non-denervated gastrocnemii to body weight*

TIME AFTER DENERVATION	Thyroxin treated			Untreated controls			Thiouracil treated		
	Tension (Nerve[1]) / Tension (Muscle[1])		Muscle weight / Body weight	Tension (Nerve[1]) / Tension (Muscle[1])		Muscle weight / Body weight	Tension (Nerve[1]) / Tension (Muscle[1])		Muscle weight / Body weight
	denervated	control	control	denervated	control	control	denervated	control	control
days	%	%	%	%	%	%	%	%	%
10–14			0.655 ±0.015			0.661 ±0.012			0.661 ±0.012
21	69.1 ±3.6	93.5 ±1.0	0.608 ±0.012	48.1 ±3.2	95.1 ±1.2	0.641 ±0.022	41.3 ±4.1	96.2 ±1.2	0.642 ±0.016
28	76.3 ±2.3	94.7 ±1.2	0.645 ±0.009	75.5 ±2.9	95.4 ±0.5	0.666 ±0.010	66.3 ±4.0	95.3 ±0.8	0.672 ±0.011
42	83.2 ±2.8	91.3 ±2.3	0.594 ±0.017	87.4 ±1.8	93.3 ±1.0	0.646 ±0.012	79.1 ±2.2	92.0 ±1.4	0.646 ±0.011

[1] Stimulation.

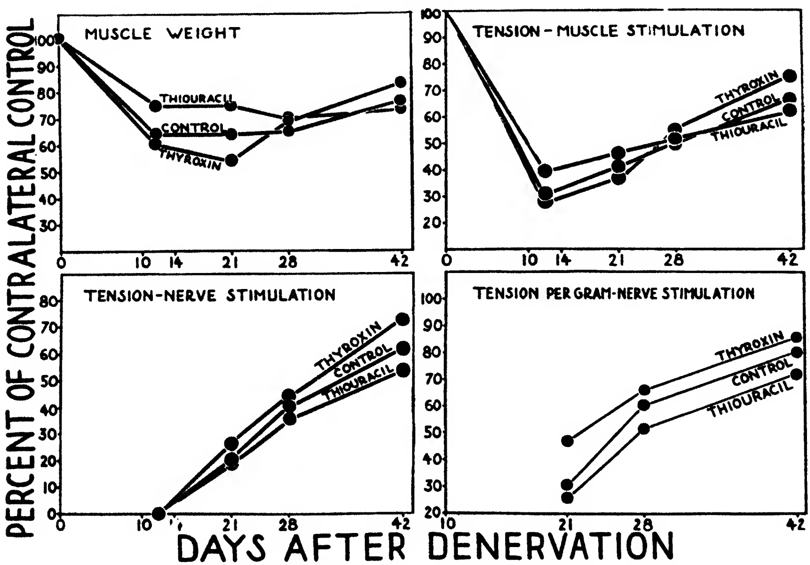

FIG. 1. A graph showing average values for the effects of thyroxin and thiouracil on neuromuscular atrophy and regeneration in the rat.

tration increased the rates of both atrophy and subsequent reinnervation and regeneration, whereas thiouracil administration was accompanied by slower rates

of both muscular atrophy and subsequent regeneration. It is to be pointed out that the studies which were made at various times after denervation permit some degree of separate analysis of atrophy and regeneration. Studies made prior to 21 days after denervation show that the denervated gastrocnemius muscles of thyroxin-treated animals were smaller and weaker than the denervated muscles of control animals. At these times atrophy was found to be retarded in the thiouracil-treated group. The velocity of neuromuscular regeneration is not revealed by the absolute or relative values for muscle weight and strength at any given time, but rather by the amount of recovery in muscle mass and strength which occurs within a given period of time during the regeneration period. By the latter criteria it is apparent that treatment with thyroxin facilitated neuromuscular regeneration, whereas the hypothyroid state resulting from thiouracil administration was associated with retarded regeneration.

Discussion. Results for atrophy and regeneration expressed as per cent of contralateral control are valid only on the assumption of the identity of the weight and strength of the contralateral muscles of an animal. This has been shown to be true for the gastrocnemii of the rat (8). Furthermore, no significant change in body weight should occur during the course of an experiment unless such changes occur equally in the control and experimental groups. The data (table 2) show that at no time was there a significant difference in either the body weight or the ratio of muscle weight to body weight between control and experimental groups. It is logical to expect that more striking effects would have been observed if larger doses of thyroxin and thiouracil had been employed. However, the fact that these drugs in large doses tend to significantly lower body weight discouraged their use in this connection.

The findings in this study are in accord with the observations of Hines and Knowlton (9) that denervation atrophy was delayed by thyroidectomy and accelerated by thyroxin administration. These findings mean that regenerating nerves make functional contacts with muscles which have undergone more atrophy in the case of thyroxin-treated animals and less atrophy in the thiouracil group than in the case of untreated control animals. In spite of this handicap the regenerating muscles of thyroxin-treated animals were stronger and larger than either those of control or thiouracil-treated animals at 42 days after denervation. In view of the fact that thyroxin does not increase the mass or strength of normal skeletal muscle, it is logical to conclude from these studies that hyperthyroidism facilitates the reinnervation and subsequent regeneration of muscle and that thiouracil administration exerts an effect in the opposite direction. In this connection it is interesting to point out that Belkin (10) has reported that regeneration of salamander limbs and wound healing in warm-blooded animals proceed more rapidly under treatment with thyroxin. The similarity of nerve regeneration to embryonic nerve growth has been pointed out by various workers (11). Denervation atrophy has been found to be correlated with the general growth rates in different species (12), and the formula expressing the rate of neuromuscular regeneration is similar to that for general body growth (13). One may then postulate that the effects of thyroxin and thiouracil treatment on neuro-

muscular regeneration are related to their effects on general body growth and metabolism.

SUMMARY

1. A study of the effects of thymectomy, thyroxin-induced hyperthyroidism and thiouracil-induced hypothyroidism on denervation atrophy and neuromuscular regeneration was carried out on albino rats.

2. In thymectomized animals studies made 4 weeks after denervation showed no influence of this condition on either normal muscle or neuromuscular regeneration.

3. In animals rendered hypothyroid by thiouracil treatment, studies made at 10–14, 21, 28 and 42 days after denervation showed a significant delay in denervation atrophy and neuromuscular regeneration.

4. Similar studies on animals made hyperthyroid by thyroxin administration showed an acceleration of denervation atrophy and a facilitation of neuromuscular regeneration. The latter appeared to be due to an earlier and more successful reinnervation.

5. It is believed that neuromuscular regeneration and growth phenomena have certain similarities, and that the effects of thyroxin and thiouracil are due, respectively, to acceleration and retardation of the components of the process.

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ON THE FORCE AND SIZE OF MOTOR UNITS IN THE RABBIT'S SARTORIUS MUSCLE

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Received for publication August 5, 1947

Eccles and Sherrington (1) determined the average force of the motor unit in cat muscles by dividing the muscle force during indirect stimulation by the number of motor nerve fibers which innervate the muscle. The latter value was found by counting the nerve fibers in the motor nerve after degeneration of the sensory fibers induced by the removal of the appropriate spinal ganglia a few months previously. The values (for faradic stimulation) found in this way ranged from about 30 grams for the average motor unit in the medial head of the m. gastrocnemius to 6 grams for the unit in the m. semitendinosus.

Clark (2) determined the average size of the motor unit by dividing the total number of fibers contained in the muscle by the number of motor nerve fibers supplying them. The units of the m. soleus of the cat consisted of an average of 120 muscle fibers; those of the m. extensor longus digitorum of 165 fibers.

Although the determination of the force and size of the normal motor unit is simple in principle, it is a problem which offers considerable technical difficulties. The following considerations are relevant:

a) In order to cause the degeneration of all the sensory fibers in the motor nerve, the appropriate spinal ganglia have to be removed in toto.

b) It has been shown that in paretic muscles the intact motor units may grow by the adoption of denervated muscle fibers (3–6). All damage to the anterior roots during the removal of the ganglia, therefore, has to be prevented, since a partial muscle denervation may result in an increase of the force and size of the units which remain innervated.

c) It is necessary to allow sufficient time after the excision of the ganglia for the removal of the myelin of the degenerated sensory fibers. However, as will be shown, regeneration of nerve fibers from various sources becomes a definite possibility if the time chosen is too long. Regenerating motor fibers will form new motor units which are likely to be abnormal in force and size. If the regenerated fibers are sensory in origin they will depress the value computed for the force and size of the motor unit, since they cannot be distinguished from regenerated motor fibers.

The conditions mentioned above are so exacting that attempts to determine in this way the force and size of the normal motor unit in the rabbit's sartorius muscle have not been successful. Instead, these values have been estimated from the force and the number of muscle fibers of the m. sartorius and the total number of nerve fibers in its motor nerve. To produce abnormally large motor units by fiber adoption, deafferenting has been combined with partial denervation of the sartorius muscle in part of the experiments.

Methods. All experiments were performed on rabbits. The muscle force of the m. sartorius was determined by recording isometrically the contraction during indirect faradic stimulation and comparing the deflection with a calibration of the lever with known weights. The initial tension was 50 grams.

The total number of fibers of the sartorius muscle cannot be determined by counting the fibers in a muscle cross section, because the fibers are considerably shorter than the muscle. The number of fibers was therefore determined by macerating the muscle for 20 hours in 20% nitric acid at 18°, and then isolating each individual muscle fiber by teasing under a binocular microscope. The heterolateral muscle was fixed in Zenker's solution, embedded in paraffin, and haematoxylin-eosin stained sections were prepared from its middle portion. In these the number of fiber cross-sections was determined.

The motor fibers for the m. sartorius are derived from the 5th and 6th lumbar segments (4). In part of the experiments the spinal ganglia of these segments were excised; in others in which deafferenting was to be combined with partial denervation, only the 5th lumbar ganglion was removed. The excision of the ganglia was performed under pentobarbital narcosis with aseptic precautions. The ganglia were exposed from the dorsal side. This was often hampered by severe venous bleeding which sometimes could not be managed, resulting in loss of the animal. After exposing the ganglia and the adjoining parts of the spinal cord, the dura was incised for about 1 cm. and the dorsal root picked up. The dura was then cut between the dorsal and ventral root. By careful teasing, the dorsal root was dissected distally until the ganglion could be separated from the ventral root. The ganglia were saved, stained with osmic acid and sectioned serially to ascertain the completeness of removal.

In the animals in which the 5th and 6th lumbar spinal ganglia had been removed, the 7th lumbar spinal nerve was pulled out of the spinal cord and at least 1 cm. of the connection between the 4th and 5th lumbar spinal nerves was resected. In addition to this, the 6th lumbar spinal nerve was pulled out in the experiments intended to combine deafferenting with partial denervation. Since the 6th lumbar segment usually provides most of the sartorius innervation, this produces a severe paresis of the muscle (4). The latter procedures were performed either simultaneous with or a few days after the removal of the ganglia. In this way connections with more cranial and caudal segments are severed, and it can be expected that all the remaining fibers in the nerve supplying the m. sartorius are motor fibers.

After recording the muscle contraction, the motor nerve was fixed and stained with osmic acid, embedded in paraffin and sectioned serially. The nerve fibers present were counted and measured with a calibrated ocular micrometer.

Results. *The number of fibers in the sartorius muscle.* The m. sartorius of the rabbit, situated superficially on the anterior and medial side of the thigh, is long, slender and quite thin. It takes its origin from the inguinal ligament, and its tendon, which is continuous with the superficial fascia of the thigh, inserts on the tibia. The muscle is innervated by one or more fine branches of the n. saphenous, a branch of the femoral nerve.

The force of the sartorius muscle during faradic stimulation of the intra-ab-

dominal portion of the femoral nerve was determined in rabbits weighing around 2.5 kgm. To prevent disturbance by the contraction of the surrounding muscles, all branches of the n. femoralis except the n. saphenous were severed. After measuring its force, the muscle was removed and used for counting of muscle fibers in the cross section.

The heterolateral muscle was removed very carefully in order to obtain the muscle undamaged, but without any extraneous muscle material. After maceration, the total number of fibers contained in this muscle was determined.

The results of 7 experiments are compiled in table 1. The force developed by indirect faradic stimulation varied between 106 and 148 grams. The mean force was 133 grams, which is in good agreement with a previous series of similar determinations (4).

The total number of fibers in the muscle varied between 7150 and 5216; the mean of 7 muscles was 6282 fibers. The weight of the sartorius muscle was of the order of 0.6 grams, and thus the average weight of the sartorius muscle fiber

TABLE 1

NUMBER	WEIGHT OF RABBIT	WEIGHT OF MUSCLE	FORCE	TOTAL NUMBER OF MUSCLE FIBERS	NUMBER OF FIBERS IN CROSS SECTION	RATIO
	kgm.	*grams*	*grams*			
1	2.6	0.60	146	7150	4950	0.69
2	2.3	—	117	5521	3429	0.62
3	2.4	—	133	5216	3303	0.65
4	2.5	0.53	145	6600	3801	0.575
5	2.6	—	—	6930	4894	0.71
6	2.4	—	148	6775	—	—
7	2.3	0.55	106	5779	3529	0.61
Mean.....	2.4	—	133	6282	—	0.64

is about 0.1 mgm., a figure well in agreement with the weights of muscle fibers computed by Clark (2) for the m. soleus and m. extensor longus digitorum of the cat.

The number of muscle fibers counted in the cross section was always markedly smaller than the number of fibers determined by teasing the macerated muscle, the mean ratio being 0.64. It was found previously (7) that the muscle fibers are considerably shorter than the muscle. In rabbits of 2.5 to 3 kgm. the muscular part of the m. sartorius is about 7 cm. The longest fibers are about 3 cm.; many are shorter. The sartorius muscle is built of three kinds of fibers: fibers attached to the muscle origin and ending freely in the muscle, fibers beginning and ending in the muscle substance, and fibers beginning in the muscle and attached to the tendon. Since no muscle fiber is longer than 3 cm., it is obvious that all the fibers cannot be counted in any one cross section of the muscle. It also is likely that most cross sections taken from the middle portion of the muscle will contain about $\frac{2}{3}$ of the total number of fibers; this agrees well with the mean ratio of 0.64 determined.

The motor nerve for the m. sartorius. There is considerable variation in the way in which the motor fibers for the m. sartorius branch off the n. saphenous. Often there are two fine nerve branches which enter into the muscle; sometimes there is only one. Occasionally this innervation is supplemented by a very fine branch which enters the muscle at a more proximal point.

In 5 experiments the nerve supply of the m. sartorius was fixed and stained with osmic acid, and after sectioning the nerve fibers were measured and

TABLE 2

	NUMBER				
	1	2	3	4	5
Fibers in motor nerve	84	108	117	70	96
Mean					95

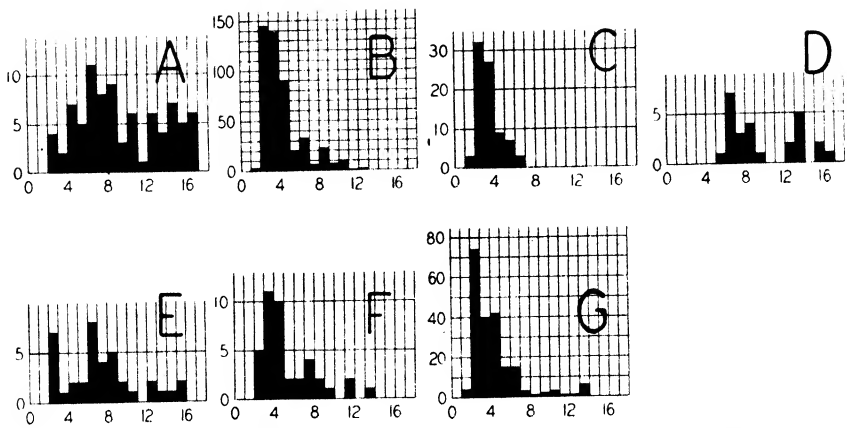

FIG. 1. A: histograms (fiber diameter on abscissa, fiber number on ordinate) of normal sartorius motor nerve; B: of a sensory branch of the saphenous; C: histogram of regenerated fibers in the sartorius motor nerve 35 days after crushing L6; D: sartorius motor nerve, deafferented 24 days before; E: the same, deafferented 31 days before; F: deafferented 53 days before; G: 76 days before.

counted. Figure 1A shows a histogram of the sartorius nerve in one of these experiments. There are few fibers of less than 4 μ diameter. Two groups of fibers can be distinguished, one with a maximum in the 7 μ range, the other in the 14 μ range. A similar distribution of fiber diameter of the motor supply was described by Eccles and Sherrington (1). Figure 1B shows the histogram of a sensory branch of the same saphenous nerve. The pronounced difference in these histograms permits identification of a branch as motor or sensory. Histograms were made of all the nerve branches used in table 2, proving them to be

motor. The number of fibers in the motor nerve ranges from 70 to 117 with a mean of 95 (table 2). Making the assumption that ½ to ⅓ of these fibers are sensory (8), the average number of motor fibers would be 50 to 60. From this figure and those of table 1 it is possible to estimate that the average motor unit of the m. sartorius consists of 100 to 125 muscle fibers, and that the force per unit is 2.2 to 2.7 grams.

Histograms of deafferented motor nerves. About 25 days after excision of the ganglia, it became possible to distinguish with certainty between intact motor fibers and myelin rests in the deafferented motor nerve. Since the distance between the spinal ganglia of L6 and the place where the motor nerve enters the sartorius muscle is about 100 mm., the earliest appearance of regenerating fibers growing from an anterior root damaged during removal of the ganglia is

$$5 + \frac{100}{4.4} = 28 \text{ days},$$

using the values of Gutmann, Guttmann, Medawar and Young (9) for the latent period of regeneration (5 days) and for the rate of growth of regenerating fibers after crushing rabbit nerve (4.4 mm/day).

To investigate this early regeneration, the spinal nerve of L6 was crushed where it emerges from the intervertebral foramen. Furthermore, the spinal nerves L5 and L7 were pulled out of the cord, and the connection between L5 and L4 was removed, isolating the crushed 6th lumbar spinal nerve from the rest of the peripheral nervous system. Intra-abdominal stimulation of the femoral nerve 35 days after this operation caused a small sartorius contraction, indicating that some of the regenerating nerve fibers had made functional connection with the muscle fibers. In the preparations of the sartorius motor nerve of this animal, 82 nerve fibers were counted, all with the thin myelin sheath characteristic of newly regenerated fibers. The histogram of the nerve shows that most of these fibers are less than 4 μ in diameter (fig. 1C). This indicates that when the anterior root is damaged during excision of the ganglia, regenerated motor fibers may be present in the sartorius motor nerve within five weeks. In some animals in which the anterior roots were accidentally crushed during the removal of the ganglia, stimulation of the femoral nerve 28 days later did not elicit a sartorius contraction, and preparations of the motor nerve were found to be free of nerve fibers. It is therefore likely that the regenerating anterior root fibers reach the motor nerve during the 5th week after the injury is incurred. It takes 3½–4 weeks before the myelin of the sensory fibers is absorbed sufficiently to identify the motor fibers, and the time during which one can be certain that all the fibers in the motor nerve are original efferent fibers is thus quite limited in a small animal like the rabbit, in which regeneration proceeds at a high rate.

Figure 1D shows the histogram of a sartorius motor nerve removed 24 days after excision of spinal ganglia L5 and L6. The serial sections of the ganglia showed that the excision had been complete, and the sensory part of the n.

saphenous was practically[1] free of fibers, which is an even better indication of the completeness of deafferenting. Since the time is too short for regeneration, it can be assumed that the 26 fibers in the motor part of the saphenous nerve are all original efferent, probably motor, nerve fibers not contaminated with sensory or regenerated fibers. The histogram shows that all fibers are more than 5 μ thick and that there are two well distinguished groups of fibers, one between 5 and 10 μ in diameter with a maximum around 7 μ, and a second group between 12 and 17 μ with a maximum at 14 μ. A comparison of this histogram with that of a regenerated motor nerve (fig. 1C) shows a profound difference in diameter distribution.

Figure 1E is the histogram of a motor nerve removed 31 days after excision of spinal ganglia L5 and L6. In the sensory part of the n. saphenous one single

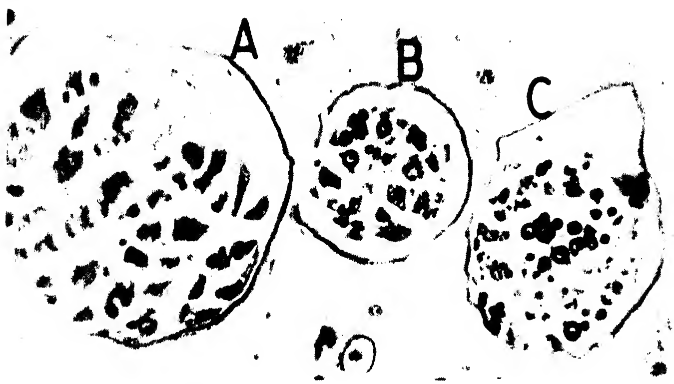

FIG. 2. Photomicrograph of part of the saphenous nerve removed 34 days after excision of the ganglion L5 and partial denervation by pulling L6 out of the cord. The figure shows the two motor branches (B and C) and a sensory branch (A) of the saphenous. The latter is like the rest of the sensory saphenous free of fibers. In the motor, branches are original and regenerated fibers.

thick fiber was present. The motor nerve contained 38 fibers. Again there was a group of fibers with a maximum around 7 μ and a group of thick fibers between 12 and 16 μ in diameter. However, a group of thin fibers has appeared, most of which have a diameter between 2 and 3 μ. They have the characteristics of newly regenerated fibers. These fibers have probably grown out from anterior root fibers damaged during the removal of the ganglia and have found their way back into the sartorius motor nerve by following the tubes of Schwann. Figure 2 shows a photomicrograph of a similar nerve.

The regenerated fibers assumed to have grown from damaged anterior roots

[1] Two intact fibers were present in the sensory saphenous, which, since they were thick and had a well-developed myelin sheath, were original fibers. They may be sensory fibers which reached the n. saphenous along some indirect route, or efferent fibers which strayed in the sensory part of the saphenous (10).

become thicker as the interval between excision of the ganglia and removal of the motor nerve is increased. The histogram shown in figure 1F is of a sartorius motor nerve removed 53 days after excision of the ganglia. The nerve contained 40 fibers. The removal of the ganglia seemed complete, and the sensory part of the n. saphenous contained only one fiber. The two groups of fibers characteristic of the motor nerve can again be recognized in this histogram. In addition, however, there is here present an important group of regenerated fibers with a maximum at 4 μ. Since these fibers are found exclusively in the motor nerve, they have to be considered as regenerated anterior root fibers.

When the time after removal of the ganglia is still further increased, additional changes occur in the histogram. Figure 1G is a histogram of a sartorius motor nerve removed 76 days after deafferenting. The spinal ganglia seemed to have been removed completely. In the motor nerve 236 fibers were present, many more than are found normally in this nerve. In the histogram, a new group of thin fibers between 2 and 3 μ in diameter is added. It seems likely that these thin regenerated fibers have grown from the nerve supply of the adjacent segments into the roots and branches of the lumbo-sacral plexus which were severed to deafferent and isolate the femoral nerve. These fibers are therefore delayed in their arrival at the sartorius motor nerve as compared with the regenerating anterior root fibers. This assumption is supported not only by the large number of fibers in the motor nerve, but also by the presence of similar thin regenerated fibers in the sensory part of the saphenous nerve. It is likely that these fibers are mostly sensory in nature.

Motor units of the m. sartorius. From the number of nerve fibers in the motor nerve for the m. sartorius it has been estimated that this muscle is innervated by 50 to 60 motor fibers. In no instance has such a number of original fibers been found in the deafferented motor nerve which seems to indicate that it has not been possible to excise the ganglia in the rabbit, without damage to the anterior roots. This is hardly surprising, as the ganglia had to be teased loose from the fragile anterior roots. In all experiments deafferenting was thus combined with partial denervation and all the units determined in this way must be considered as abnormal. Therefore, no distinction will be made between the experiments in which the m. sartorius was deafferented intentionally and the others in which this occurred accidentally.

Data from 10 experiments selected from a large number have been compiled in table 3. The motor nerves of the first three experiments contain only fibers thicker than 5 μ (histograms 1D, 3A and 3B), which identifies them as original efferent fibers. The average force per motor unit in the first experiment is 1.7 grams, a value somewhat lower than the estimated tension of the normal motor unit. The number of motor fibers is 26, about half that of the estimated number. Growth of the motor unit by fiber adoption would thus have been possible; the small force of the motor unit indicates its failure in this particular experiment. In experiment 2 (table 3) the tension produced by the unit (5.3 grams) is about twice that of the estimated force of the normal unit, and in experiment 3 it is 3–4 times as large (8.8 grams). In the latter experiments,

fiber adoption seems to have been active, especially in experiment 3 where the small number of intact nerve fibers favors the growth of the motor unit (4).

The histogram shows the presence of regenerated fibers in the other experiments of table 3. Probably only one such fiber was present in the motor nerve of experiment 4 (histogram 3C). The force of the motor unit in this experiment was 4.0 grams. In expèriments 5, 6 and 7 (histograms 3D, 1E and 3E), there are more regenerated fibers, which probably originate in the anterior roots, since the

TABLE 3

NUMBER	GANGLIA EXCISED	TIME FOR DEGENERATION	NUMBER OF NERVE FIBERS IN MOTOR NERVE	HISTOGRAM	MUSCLE FORCE	FORCE PER UNIT
		days			*grams*	*grams*
1	L5–L6	24	26	1D	43	1.7
2	L5–L6	32	19	2A	100	5.3
3	L5	34	5	2B	44	8.8
4	L5	28	12	2C	48	4.0
5	L5–L6	32	39	2D	102	2.6
6	L5–L6	31	38	1E	46	1.2
7	L5	42	31	2E	98	3.2
8	L5–L6	55	27	2F	134	5.0
9	L5	73	23	2G	108	4.7
10	L5	125	15	2H	81	5.4

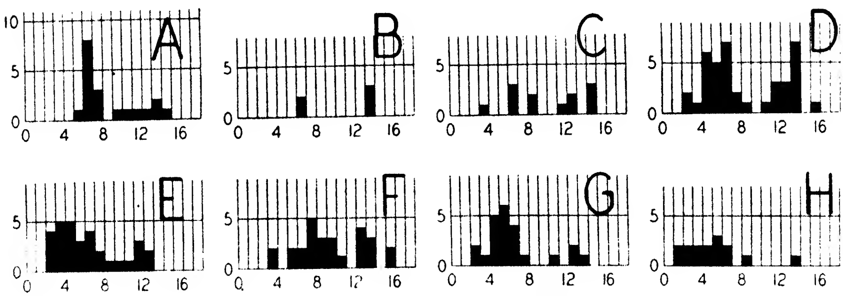

FIG. 3. A: histogram of the sartorius motor nerve; deafferented 32 days before; B: the same deafferented 34 days before; C: deafferented 28 days before; D: 32 days before; E: 42 days before; F: 55 days before; G: 73 days before; H: 125 days before.

sensory parts of the saphenous nerves were free of nerve fibers (except for a single fiber in the sensory saphenous of experiment 6). The regenerating motor fibers probably have not had time to form large motor units, as the time between removal of the ganglia and the final experiment was only 30 to 40 days. In these experiments abnormally large units due to fiber adoption may be present, as well as regenerated units which are smaller than normal. The units in these three experiments produced tensions of 2.6, 1.2 and 3.2 grams, respectively, which may illustrate the action of these conflicting influences.

In experiments 8, 9 and 10 (histograms 3F, 3G and 3H) in which the time after excision of the ganglion was 55, 73 and 125 days, the total muscle force (134, 108 and 81 grams) approached that of the normal muscle. The growth of the original units by fiber adoption and the regeneration of new motor units, which by that time may be as large or even larger than the normal ones (11) has resulted in a more or less complete reinnervation of these muscles, although the number of fibers in their nerve supply is considerably below normal. The average force computed for this mixture of units was respectively 5.0, 4.7 and 5.4 grams, or about twice the estimated force of the normal unit. It is likely that the actual force of these motor units is larger, however, since at this time a new group of regenerated fibers usually appears, which as mentioned above are probably mostly of sensory origin. Such fibers were present in the sensory part of the saphenous in these experiments.

DISCUSSION. The determination of the force of the motor unit of the rabbit's sartorius muscle has been found to be a difficult problem. The removal of ganglia L5 and L6 in general produced damage to the anterior root, causing partial denervation of the muscle, resulting in the adoption of denervated muscle fibers by the intact motor units. On the other hand, after such damage regeneration of the anterior root fibers occurs, adding regenerated and thus abnormal units to the oversize units due to fiber adoption. Finally nerve fibers, probably mostly sensory, may grow in from adjacent segments, further complicating the picture. These factors account for the great variation in the computed values for the force of the motor unit (ranging from about 1 to 9 grams).

Perhaps the most reliable figures for the force and size of the normal motor unit are those estimated from the mean force and number of fibers of the normal muscle, and the mean number of nerve fibers in the motor nerve. These values were respectively 2.2–2.7 grams and 100–125 fibers. Comparing these with the figures of table 3, there seems to be no doubt that growth of the motor unit by fiber adoption can increase the size of the unit materially.

Eccles and Sherrington (1) worked on more caudal segments of the cat, where the conditions for the removal of the spinal ganglia without injury to the anterior roots seem to be more favorable. They found thin fibers down to 2 μ in the deafferented motor nerves. In the present experiments on rabbits, no such fibers were found in motor nerves, which could be assumed to be free of sensory or regenerated fibers. Eccles and Sherrington argued that these thin fibers are of anterior root origin. In view of the long period of degeneration used by these authors, it seems possible that at least some of the thin fibers are regenerates.

The force developed per muscle fiber, calculated by dividing the mean muscle force (133 grams) by the mean of the total number of muscle fibers (6282), is about 22 mgm. This figure is smaller than the values for cat muscles computed by Clark, 48.5 mgm. per fiber for the m. extensor longus digitorum and 84 mgm. for the m. soleus. However, the arrangement of the fibers in the m. sartorius is such that three muscle fibers are placed more or less serially, forming as far as function is concerned one long fiber stretched over the entire length of the muscle. This arrangement will increase the extent of motion, but the force of

three such fibers will not be any larger than that of a single one. The actual force of the muscle fiber thus will be about three times as large as that computed above. The force per muscle fiber of the m. sartorius would be about 66 mgm., a figure more in agreement with the values found by Clark.

The values estimated for the force of the sartorius motor unit (2.2–2.7 grams) is small as compared with Eccles and Sherrington's figures for the units of some cat muscles (6 to 34 grams). The number of muscle fibers in the sartorius unit is of the same order as found by Clark (2) for cat muscle. Due to the shortness of the sartorius motor nerve, the fiber counts were performed at a distance of about 10 mm. from the muscle. At this point some fiber division has probably occurred (Eccles and Sherrington, 1930), which increases the fiber count and thus decreases the computed force and size of the unit. However, from Eccles and Sherrington's figures it seems that this factor cannot account for the large difference between the force of the units in the rabbit sartorius and in the cat muscles. It is likely that the smallness of the force estimated for the sartorius units is again mainly due to the arrangement of the muscle fibers in series of three, which span the length of the muscle. Taking this into consideration, the force of the sartorius units agrees rather well with the values found by Eccles and Sherrington (1) for some of the cat muscles (m. semitendinosis, m. soleus and m. extensor longus digitorum).

SUMMARY

1. In seven sartorius muscles of the rabbit the total number of muscle fibers was determined. The values found were between 5216 and 7150 fibers; the mean was 6282 fibers. The mean force developed by the heterolateral sartorius muscles was 133 grams.

2. In 5 experiments the nerve fibers in the motor nerve of the m. sartorius were counted. Figures between 70 and 117 were determined with a mean of 95 fibers.

3. Assuming that $\frac{1}{3}$ to $\frac{1}{2}$ of the fibers in the motor nerve are sensory, the mean sartorius motor unit can be estimated to consist of 100 to 125 muscle fibers, producing a tension of 2.2 to 2.7 grams during faradic stimulation.

4. In the histograms of sartorius motor nerves which had been deafferented by excision of the appropriate spinal ganglia, and which could be expected to be free of sensory and regenerated fibers, all nerve fibers were more than 5 μ in diameter. Two groups of fibers were present, one with a maximum around 7 μ, the other with a maximum around 14 μ.

5. Regenerated fibers originating from an anterior root damaged during removal of the ganglia may be present in the sartorius motor nerve within 5 weeks. Later (usually after 70 to 75 days) regenerated fibers which are probably mostly sensory may grow into this nerve from other sources.

6. Complete deafferenting, without damage to the anterior roots, and absence of regenerated fibers in the motor nerve are conditions for the determination of force and size of the motor unit. These conditions are so exacting that they have in the present experiments never been met. In most experiments the motor

unit produced considerably more tension (up to 8.8 grams) during faradic stimulation than the value for the normal unit estimated above (2.2–2.7 grams). This is probably due to fiber adoption of the intact units in muscles partially denervated during the removal of the ganglia.

I am indebted to Mrs. J. Wiersma for valuable assistance and to Miss R. E. Estey, who patiently counted many thousands of muscle and nerve fibers.

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EFFECTS OF DIISOPROPYL FLUOROPHOSPHATE (DFP) ON ACETYLCHOLINE STIMULATION OF THE FROG RECTUS ABDOMINIS MUSCLE[1]

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Received for publication August 9, 1947

Response of the isolated rectus abdominis muscle of the frog to acetylcholine stimulation is markedly potentiated by increased acidity of the ambient solution. Carbon dioxide, lactic acid, phosphoric acid and hydrochloric acid all produce increased response, the extent of which seems to be dependent upon the penetrating powers of the individual acids (1). Carbon dioxide is the most effective of these acids, presumably due to its rapid penetration, so that it easily reaches the intracellular areas where acetylcholine is thought to exert its effects. The more fixed acids, such as phosphoric and hydrochloric, are less effective, exerting comparable effects to those of carbon dioxide and lactic acid only at much lower pH values.

During experiments which demonstrated that diisopropyl fluorophosphate (DFP) also potentiated the response of the rectus abdominis of the frog to acetylcholine, it was observed that solutions containing DFP were extremely acid in character. Since previous work has shown that any increase in acidity of the ambient solution has a potentiating effect upon this response, it seemed reasonable to postulate that at least part of the action of DFP might be due to its acid properties.

Rectus abdominis muscles of the frog were excised, mounted in an especially devised container and attached to a recording muscle lever. The container was equipped with a three-way stopcock for quickly flooding and changing the solutions about the muscle. Bicarbonate-free Ringer-Locke solution was used as the medium to which acetylcholine and the test solutions were added. The muscle was flooded for one minute with acetylcholine-containing solution, quickly drained and reflooded with bicarbonate-free Ringer's for a 10-minute rest period, followed by another flooding with acetylcholine plus the test solution (DFP, CO_2, H_3PO_4). The hydrogen ion concentration of each solution was determined with the glass electrode.

The effect of DFP in buffered and in unbuffered solutions is shown in figure 1. 1A, C and E are control contractions in response to 1:200,000 acetylcholine in bicarbonate-free Ringer-Locke solution at pH 6.4. 1B is a contraction in response to the same concentration of acetylcholine in bicarbonate-free Ringer-Locke solution to which DFP has been added to a concentration of M/2500. A 64% increase in height of contraction occurred under these conditions. The

[1] These experiments were supported in part by a grant from the Horace H. Rackham School of Graduate Studies, University of Michigan.

same concentrations of acetylcholine and DFP were added to a standard Ringer-Locke solution to produce the 20% increase in height of contraction shown in 1D. The only difference in conditions between the two test stimulations is that in 1B the acidity of the DFP was not buffered and the pH was reduced to 3.3, whereas in 1D the presence of sodium bicarbonate in the standard Ringer-Locke solution buffered the DFP so that the pH was 6.3.

Experiments such as this suggest that the increase in contraction of the muscle caused by the presence of DFP is a resultant of two factors: first, a function of the acidity of the solution, and second, a response to some specific anticholin-

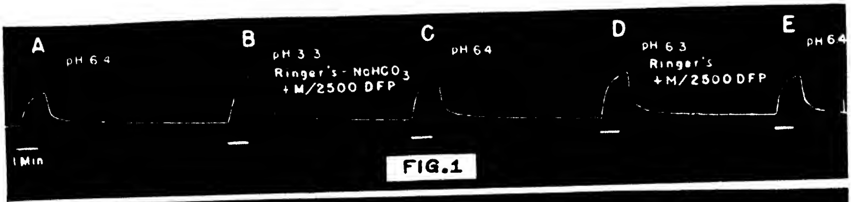

FIG. 1

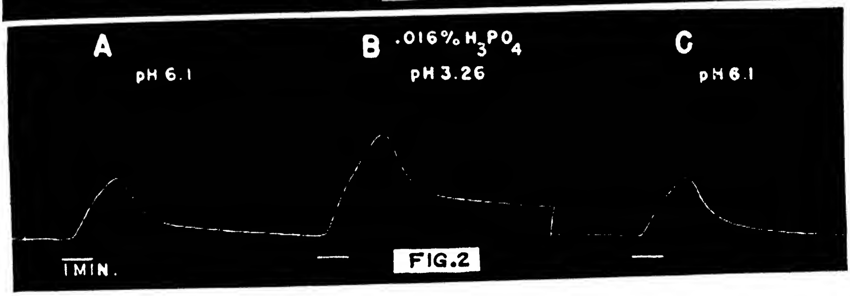

FIG. 2

FIG. 1. Response of the isolated rectus abdominis muscle of the frog to acetylcholine (1–200,000) modified by diisopropyl fluorophosphate (DFP), in unbuffered and in buffered Ringer-Locke solutions. A, C, and E are control contractions in bicarbonate-free Ringer-Locke solution. B is the response to addition of DFP to a concentration of M/2500 in the bicarbonate-free solution. D is the response to addition of DFP (M/2500) to a standard Ringer-Locke solution.

FIG. 2. Potentiation of response of the isolated rectus abdominis muscle of the frog to acetylcholine (1–1,000,000) by phosphoric acid. A and C are control contractions in bicarbonate-free Ringer-Locke solution. B is the response to addition of 0.016% phosphoric acid to the ambient solution.

esterase activity of the compound. These results are in agreement with the additive effects of physostigmine and carbon dioxide previously described (2).

Since DFP is closely related to phosphoric acid, a comparison was made of the responses of the rectus abdominis to DFP and to H_3PO_4. Figure 2 demonstrates the response of a rectus abdominis muscle to 1–1,000,000 acetylcholine in the presence of .016% H_3PO_4, with a pH value of 3.26. The increase in height of contraction is similar to that shown in figure 1B, which is at a comparable pH.

Another example of similarity in response of the rectus abdominis to DFP and to phosphoric acid is that when high concentrations of acid are used, or repeated

exposures are made, there is a maintained increased height of contraction. This is suggestive that acid, like DFP, may have a relatively irreversible effect in high concentrations.

It is therefore obvious that the acid properties of DFP should not be overlooked in the interpretation of its physiological effects in *in vitro* experiments such as described in the present paper. On the contrary, these readily demonstrable acid effects should not be confused with the powerful specific anticholinesterase effects of DFP noted in mammalian experiments where approximately 7 mgm. of DFP injected into dogs weighing approximately 14 kgm. produces striking potentiation of nervous activity. In consideration of the great buffering capacity of a dog of this size, the physiological effects of DFP obviously cannot be attributed to its acid properties.

Solutions of DFP deteriorate rapidly and in the process become more acid in character. Experiments suggest that the specific anticholinesterase properties of DFP solutions are reduced rapidly after preparation, whereas its anticholinesterase activity as an acid becomes increased.

SUMMARY

Response of the isolated rectus abdominis muscle to acetylcholine is potentiated by DFP. Part of this effect is held to be due to the acid properties of DFP since potentiation of response to acetylcholine is considerably greater in an unbuffered solution of DFP. It is therefore suggested that the anticholinesterase activity of DFP is mediated by two effects: *a*) the acidity of DFP due to its structural similarity to phosphoric acid and *b*) the specific anticholinesterase activity of DFP itself.

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URGES TO EAT AND DRINK IN RATS

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Received for publication August 14, 1947

Studies of nutrition usually depend upon the animal's urges to eat and to drink. But little has been done to find what factors of the diet modify those urges. When body weight is employed as a measure of nutritive state, the observer ordinarily is assuming that intake has been sufficient to supply whatever is available. If weight has been lost he often assumes that the diet is inadequate qualitatively and not quantitatively. To what extent is this confidence in the urges toward maintenance justified?

The following aspects of intake are here investigated: effect of dilution of food by roughage, effect of flavoring, effect of dilution of food by water, forcing of water intake by mixture of water with food, and after-effects of partial or complete privation of food, or of water, or of both. The investigation was designed to ascertain how an animal solves conflicts, and practices priorities, in its bodily maintenance. If it be obliged to ingest an excess of water while obtaining food, how much excess will it take? If more than the requirement of some constituent cannot be avoided, will the total intake be in excess of metabolic uses? In general, how much excess of A in the diet will reduce or stop the intake of B? How are conflicts of metabolisms compromised, and do the urges of intake correspond to a pattern that favors maintenance of the individual, without or with the connivance of the machinery of outputs? By answering such questions, even in part, the physiological organization of the animal body can be partially described. In a general way it is recognized (1) that mammals often gauge intakes according to certain nutritive values, in spite of inequalities of form and substance in various diets. But it is also known that some constituents of food or drink act as deterrents to ingestion, as is illustrated by substitution of sea water for fresh water (2).

The investigation included attempts to induce rats to ingest large quantities of water, simply by mixing the water with the food available to the animals. As a consequence, a general method was worked out of forcing into the metabolism of an animal considerable quantities of various ingested materials that would ordinarily be refused.

Help in the experiments was received from M. C. Nudo, S. Parmington and M. M. Stiler. Aid in completing the investigation was derived from a contract between the Army Air Forces and the University of Rochester.

Mixtures of food with roughage. What characteristics of foods may guide rats' food consumption? Are the textbooks correct in suggesting that alimentary fill is a chief requirement of the animal? Male white rats were furnished first with an adequate dry food (chow) ad libitum, then later given the same food thoroughly mixed with a form of roughage (cellulose or kaolin). Water was

separately available. The food consumption and the water consumption were measured in 24-hour periods.

Of a constant sample of food, the consumption was highly uniform. Thus, chow was taken by one individual rat to the extent of 6.1% of the body weight each 24 hours (mean of 94 days), water being available ad libitum. The amount of food taken daily varied in this rat by ±0.65% of the body weight, hence its intake had a coefficient of variation of ±10.7%. The variation was no greater among 7 individuals (table 1) than it was in 1, providing male rats of 150 to 300 grams weight were alone considered.

Cellulose. Celluflour is reported (3) to contain no utilizable material, to be 79% cellulose and 17% pentosan. The chow is stated by its manufacturer to contain 4% of crude fiber, 12% of water and 23% of protein.

TABLE 1. *Intakes of two foods by rats*

The same 7 male individuals were given each food for 77 and 114 rat-days, respectively.

	DRIED WHOLE MILK	CHOW
Potential energy of food	5.1 Cal./gram of food	3.3 Cal./gram of food
Food consumed/day	4.0% of body wt.	5.59% of body wt.
Potential energy consumed/day	20.4 Cal./100 grams of wt.	18.6 Cal./100 grams of wt.
Protein consumed/day	1.08 grams % of wt.	1.31 grams % of wt.
Water drunk/day	14.56% of body wt.	12.18% of body wt.
Water drunk/food consumed	0.71 ml./Cal.	0.66 ml./Cal.
Water content of food	2%	12%
Water of oxidation + content in food	2.54% of wt./day	2.75% of wt./day
Total water available	0.84 ml./Cal.	0.80 ml./Cal.

When the chow was diluted by adding diverse proportions of powdered celluflour to it, the total bulks eaten increased or decreased (fig. 1), depending upon the mixture. Only in the middle range of nutrient proportions (25 to 33% nutrients) was the transition gradual; in others the whole transition was made on the first day of the new diet. The mean quantities eaten varied chiefly with the extent of dilution (fig. 2). If the object of a regime be to force the ingestion of cellulose, the optimal food mixture would evidently be about 33% nutrients. In general, a food mixture furnished to the rat a diminished stimulus to eat bulk whenever the utilizable material was less than 25% of the dry weight. When no nutrient was added to the cellulose, practically none was eaten.

The body weight was maintained only in the richer mixtures of food (fig. 2). At 33% of nutrients or less, the intake of nutrients was clearly inadequate and weight was steadily lost. The loss of weight was at the same rate as when the food intake was limited (in paired feedings) to the amounts of undiluted food actually obtained. It may be supposed that the rat was partially limited by

its alimentary capacity from handling every day more than one-fourth of its body weight of bulk (solids plus water) and one fourteenth of its weight of roughage. But below it will be shown that when the bulk is mainly composed of water, five fold as much total ingesta are taken. Factors other than bulk are believed to participate in limiting the intake.

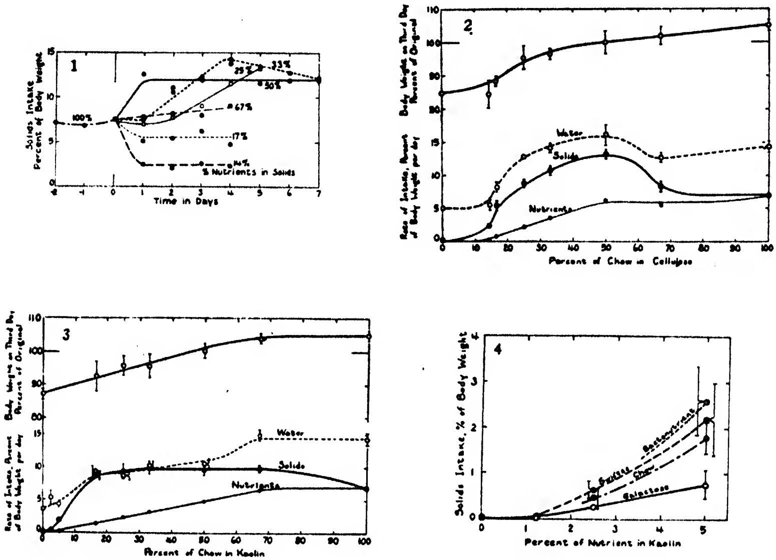


FIG. 1. Daily ad libitum intakes of solids by rats transferred at zero day from chow diet to chow mixed (in various fractions) with powdered cellulose. Each point is the mean of 3 to 5 tests; each line is drawn to fall within the standard error of all the points that belong with it. In this and subsequent graphs each intake is represented at the end of the 24-hour period to which it applies.

FIG. 2. Daily intakes of available nutrients, solids, and water, and changes of body weight, in rats maintained on various mixtures of chow and cellulose. Each point is the mean of 12 to 24 days, and the standard errors among these days are represented by the vertical lines.

FIG. 3. Daily intakes and changes of body weight in rats maintained on various mixtures of chow and kaolin.

FIG. 4. Effect of adding small quantities of flavored nutrients to kaolin, upon the daily acceptance of the kaolin mixture. This figure also amplifies the left-hand portion of figure 3.

There appears to be evidence that in the rat's alimentary tract some cellulose is decomposed (4). The products of decomposition are not such as to furnish nutrients to the rat, however. The usual assumption is that among mammals only ruminants derive energy from ingested cellulose, since they alone are believed to harbor symbionts that digest cellulose to utilizable sugars.

Kaolin. For comparison, another diluent of the food, kaolin, was chosen as one from which no utilization of energy was possible. When diverse mixtures

of chow and kaolin were presented, increased bulks were again eaten. Approximately the same relations of amounts ingested to concentrations of nutrients were found (fig. 3) as for cellulose. Slightly more kaolin was accepted in the region of 15% nutrient and slightly more cellulose at 50% nutrient. But for both the maximal roughage ingested in one day was one-fourteenth of the body weight.

It may be concluded that the proportion of nutrients in a food mixture plays a predominant role in eating, and that cellulose as a non-nutrient is not preferred to clay. Within limits, rats eat for calories.

It is notable that from dilutions of food with roughage, less nutrient material was taken than from undiluted food. To this small degree, bulk of food is a factor. Or it may also be said that only of concentrated food did the rat eat more than it needed for maintenance of body weight. In fact, for the equilibrium of weight a food of low nutrient concentration (50% utilizable) was sufficient. But to increase the body weight a highly concentrated nutrient was apparently required. In natural conditions also it is likely that a rat does not maintain its weight on a food mixture whose nutritive content is less than half. A high fraction of utilizable material is widely understood to be an important factor in edibility and acceptance. The above experiments demonstrate that this factor constitutes a necessity and not a mere preference.

Cellulose as diluent leads to more consumption of water than kaolin does. Probably the cellulose swells as it enters the alimentary tract. With kaolin mixtures the water consumption was linearly related to consumption of nutrients in the same manner as with direct partial privation of food.

It might be supposed that the ingestion of roughage could be increased by previous privation of food. This was not the case. In general, as shown below, rats deprived of food but not of water for 1 to 6 days consumed about the same amount of food per day after privation as they did before privation. So also, when food mixed with roughage, containing 33, 50, or 67% of nutrients, was offered before and after 3 days of privation, no individual ate more in 1 day subsequently than previously. Inanition is not a significant stimulus to subsequent ingestion of more roughage-containing food in the rat.

In numerous instances the rats attempted to scatter the diluted foods from the food cups, whereas they made no attempt to scatter concentrated ones. In the present tests deep jars were used as food cups so that scattering was rarely possible. In deep jars the rats often burrowed through the food mass, churning it over, as though hunting for more concentrated portions within it. Low nutrient concentrations evidently induce overt action in ways other than that of eating more; the action indicates that the low quality of the food is recognized before ingestion.

Flavors. In addition to the factors of bulk and of utilizable nutrients, some factors of taste were tested. Food acceptance of an adequate diet is often believed to depend upon tastes and odors; yet the amounts of an adequate food mixture taken by rats are influenced only to moderate though significant extents by added substances (5). The question in our experiments was: Could a rat be

induced to ingest non-nutrients when they were suitably flavored? Some of the flavoring materials tested had no nutrient value; others contained considerable nutrient. The experiments show that flavors of the first sort (diacetyl, butyric acid) induced no more eating than their absence, though saccharin did lead to a barely significant increase in eating. Hence it seems difficult to fool a rat into accepting a non-nutrient by such means. Flavors that furnished nutrients in small amounts induced eating in close proportion to their nutrient contents (fig. 4), taking chow as the standard of comparison. It is still possible that flavors may make a greater impression where choices among foods are allowed. It is also possible that more impelling flavors will be found.

Kinds of food. Two standard foods were compared, dried whole milk and chow. The two have very different contents of potential energy (table 1), the dried milk yielding 3/2 as much energy per gram of weight as the chow. When the voluntary consumptions of the two foods were compared, the richer in energy was ingested in smaller amount. The Calories actually consumed ad libitum turned out to be slightly greater in the richer food. The water consumed was proportional to the energy supplied by the food eaten. The dried milk is richer by virtue of its higher fat content, the protein representing 21% of the potential energy in milk and 28% in chow. Again it is suggested that the bulk ingested played a moderate role, but that the principal guide to the amount of ingestion was the caloric value.

The transition from one food to the other was characterized by a significant change of body weight. In passing from chow to dried milk, 2.7% of the weight was lost in 24 hours. In passing from milk to chow, 3.8% was gained. The difference between 3.8 and 2.7 corresponded roughly to the daily weight gain on constant diet. The change of weight represents, probably, the increased alimentary fill that prevails on the chow diet, since it remains longer in the alimentary tract.

SUMMARY. Mixing of diverse materials with adequate nutrients is, it appears, a method of forcing the ingestion of substances that are ordinarily refused. In other researches the method has been used incidentally for rats; as an example, Gamble *et al.* (6, 7) forced urea and various salts, in amounts that constituted up to 18% for sodium chloride and 36% for urea, of the weight of food eaten. In each of these instances the full standard quantity of food as measured in terms of its potential energy was ingested.

The conclusion from the above experiments is that both bulk and nutritive value are factors in food consumption. But a compromise between them is regularly made, according to a pattern that is uniform among many individuals. Satiety makes itself felt both in the alimentary tract and in the metabolizing tissues, and both factors then influence the motor activity of food ingestion.

Mixtures of food with water. A further development in the study of mixtures of non-nutrients with nutrients concerned the dilution of food with water. Would a rat increase its water intake to any lengths in obtaining food? At what point would mixtures be treated by the rat as though they yielded diminishing returns? Would there be any relation between water intake and the capacity for excreting water?

For this study milk fortified with copper, iron and manganese (8) was the sole diet. It was furnished ad libitum either dry (klim), or in blended dilutions of the same, or in equivalent dilutions of a uniform condensed milk (formulac), or of fresh cow's milk. Tap water was always available. Whereas the dried milk was presented in a deep beaker, the liquid milk was furnished in an inverted flask or cylinder with drinking tube. The outlet was usually arranged so that any spilled food did not enter the urine funnel. Care was taken to present the same drinking tube to any one rat each day; the cylinder was refilled with fresh materials each 24 hours and sometimes twice daily. Sodium benzoate (0.2%) also prevented coagulation and separation of the milk which stood at 26°C.

In the first 24 hours on diluted food the rats took nearly, but not quite, as large amounts of it as in subsequent days (fig. 5). They therefore lagged slightly in adjusting to the diluted food. They continued to take uniform amounts for at least 6 days, except that of the lowest dilutions (0 and 0.65% solids) less was taken after the third day. Therefore, only the extreme dilutions offered any progressive discouragement to ingestion. The amounts ingested were accordingly averaged for each entire period of 3 to 6 days.

The quantities ingested were ascertained for the various dilutions of food indicated in figure 6. Three sets of experiments are represented: *a*) dilutions of klim during 2 days, *b*) dilutions of formulac during 6 days and *c*) dilutions of formulac, each lasting 3 days, presented in uninterrupted series progressing from 5.2% solids down to 0.65% solids.

The amounts of liquid taken by the rats increased progressively with dilution of the food from 30% solids down to 2.6% solids. With further dilution less was taken, and with tap water alone the ingestion was small indeed. Of 2.6% dilution the rats were regularly ingesting more than their own body weights of water every 24 hours.

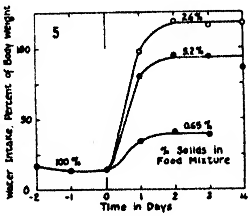
Correspondingly, the urinary output was greatly augmented by food dilution (fig. 6). In the presence of the dilution that was ingested most copiously, each rat was excreting its body weight of water every 24 hours. Evidently food dilution constituted an easy method of increasing enormously the turnover of water. The greatest output was obtained by furnishing to the rat only 2 to 3% of solids in the food.

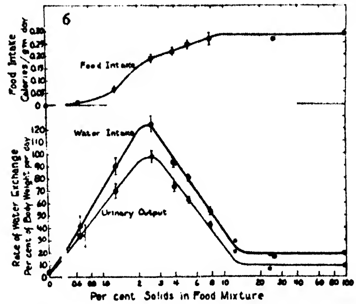
The difference between intake of water and output of urine is not an accurate measure of evaporative losses of water, since variable amounts of urine were lost during its collection. On the average the urine collected was 78% of the water ingested. In these tests, water intake appeared to be the sole determiner of urinary output. The concentrations of the urine excreted reflected the great dilution of the excretory substances by the large volumes of water being put out (fig. 7).

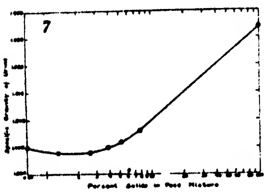
If the rat was to obtain the same quantity of nutrients other than water, it would have to double its intake every time the concentration of mixture was halved. It almost did so from mixtures containing 15% to 2.5% of solids. Of smaller concentrations it obtained less and less food, as shown in the upper portion of figure 6.

Nevertheless, the body weight was approximately maintained upon the smaller

caloric intakes down to a concentration of 2.6% of milk solids, though only after a slight loss of weight in the first 2 days. Upon lesser concentrations the body weight was not maintained; the two smallest concentrations yielded little advantage over pure water.







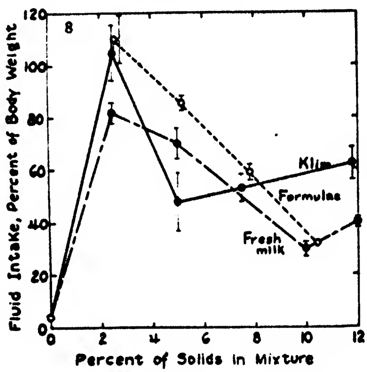


FIG. 5. Daily ad libitum water intakes of rats transferred at zero time from solid milk to eat, plus water to drink, to milk diluted with three different proportions of water. Each point is the mean of 10 tests in the uppermost curve, and of 3 tests in the other 2 curves.

FIG. 6. Daily exchanges of available nutrients, ingested water and urine in rats maintained, each for 3 to 6 days, on various concentrations of milk. A logarithmic scale is used for the concentrations of the food mixture. Free water was always present in addition to the food mixture. Each point is the mean of 18 to 36 days, with its standard error.

FIG. 7. Mean specific gravity of urine in relation to rate of water intake when food (milk) was diluted to various extents.

FIG. 8. Daily intakes of water from three kinds of milk preparations in similar dilutions.

It seemed possible that gradual and progressive dilution of the food with water (set C) would lead to some sort of adjustment in intake and body maintenance. No such adjustment was noticable, however, and the same amounts of liquid food were taken of each concentration as when the rats were suddenly transferred to each of the concentrations (sets A and B). In fact, no simple method was found of modifying the amount of each dilution that the rats accepted.

Body weight did not increase appreciably during the transition from pure

food to diluted food. Hence the retained water was insufficient to detect in terms of body weight as ascertained at daily intervals. Comparison of three preparations of milk in equal dilutions showed no consistent differences of water intake among them (fig. 8).

Limiting factors. What is the significance of the maximal rate of water intake? Is it feasible for the rat to ingest still more of the dilute food and thereby to maintain its weight? The amount taken might be limited by the alimentary tract, or by the excretory system, among other possibilities. Within the alimentary tract, digestion of the proteins or fats might be limiting, thereby making the nutrients unavailable in dilute foods. Or absorption from the alimentary tract might reach a maximal rate. That absorption of the water was not limiting in the present tests is suggested by the fact that no more diarrhea prevailed when rats ingested diluted milk than when they ingested dried milk.

TABLE 2. *Maximal rates of water exchange in adult rats*

Numbers of animals or tests are shown in parentheses.

	% OF BODY WEIGHT PER HOUR	
	mean	*maximum*
Maximal water *ingestion*		
Hypophysial injury (Richter)	4.5 (7)	5.0
Food dilution to 2.6% solids	5.2 (32)	10.0
First hour after 48-hours' water privation	7.5 (12)	10.1
First hour after 48-hours' of M/2 NaCl	6.7 (8)	12.4
Maximal water *excretion*		
Hypophysial injury (Richter)	3.9 (7)	
Food dilution	4.1 (32)	7.5
Water by stomach (Dicker and Heller)		6.2
Hourly water by stomach (Richter)	4.2 (4)	5.9
Hourly water by stomach and epinephrine under skin (Gaunt *et al.*)	5.1 (14)	

If the excretory system is limiting, then it might be that no faster excretion of water occurs under other circumstances than under the present ones. Richter (9) found that rats exhibiting diabetes insipidus after injury to the hypophysis had a highest continued rate of water intake of 120% of the body weight in 24 hours (table 2). Since rats given excesses of water by stomach tube excreted an average of 101%, and up to 140%, of their weights of collectable urine per 24 hours (though this test did not continue for 24 hours), Richter suggested that the excretory capacity for water was the limiting factor in the water turnover of diabetes insipidus. Rates up to 148% per 24 hours (not continued for 24 hours) have been reported by Dicker and Heller (10). We have sometimes collected 200% of the body weight of urine in 24 hours, the highest recorded being 240%. Even that rate is far below the believed glomerular filtration rate of the rat, which amounts to 500% of the body weight per 24 hours as inferred from inulin clearance (10), a clearance which itself appears not to vary in the rat with rate of water excretion.

Unilateral nephrectomy did not diminish the ingestion or the excretion of water below that of control individuals except to halve the turnover upon the day of operation. Tests of this sort will be described in a later report, together with the small amounts of hypertrophy of the kidneys that are initiated.

It can be concluded that the rate of water turnover in rats is not limited by any single known factor.

A rat maintained on ordinary dry food spends about one-twentieth of its time in eating and drinking. On the mixture containing 2.6% of milk solids, the rat can be observed to spend as much as one-fourth of its time in drinking. The actual ingestion, therefore, comes to be a serious amount of physical activity, even in an animal that has nothing else to do.

Dilution of food by water tells the same story as dilution by non-nutrients. The rat's acceptance is apparently guided for the most part by the nutritive quantities. Its intake of bulk is increased in moderate dilutions. But in high dilutions the compromise with bulk is diminished with the futility of the intake. The qualitative similarity with dilution by roughage prevails in spite of the fact that with roughage the diluent does not pass beyond the alimentary tract, while with water both absorption and urinary excretion of the water are concerned in its disposal.

Interrelations of food intake and water intake. The experiments described below were designed to reveal the rat's ingestive responses to *a*) forcing of concentrated food, *b*) privation of food for various periods, *c*) privation of food and water for various periods and *d*) privation of water for various periods. Remarkably reproducible effects are apparent both during the periods of unusual availability and subsequent to them. They reveal additional features of the rat's regulations of supply.

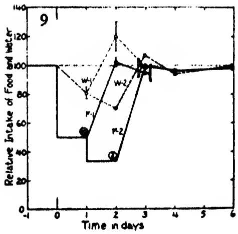
Excesses of food. In a few experiments food was forced upon the rat by stomach tube. The most concentrated food that was used had 38 grams of milk solids in 100 ml. of food. When 90% of the control daily nutrient intake was forced upon 5 rats by stomach tube in two portions during one day, other food was practically refused for the remainder of the 24-hour period. On the two subsequent days, 73 and 84% of the control intake was taken respectively.

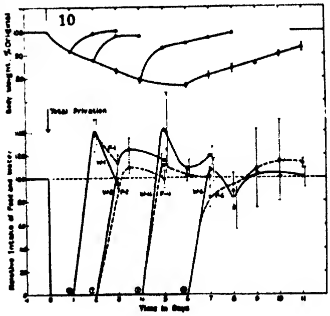
The same refusal to eat occurred after forced intake following a day of food privation. The administration of food by stomach tube inhibited the eating, whereas water similarly administered did not affect food consumption.

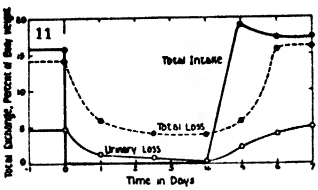
Administration of foods by intraperitoneal injections was not satisfactory. Concentrated solution of glucose and of protein digests were tolerated only in small amounts, and continuous flows of nutrient solutions would have been necessary if the number of injections was to be reduced to a reasonable standard. Nevertheless, injected nutrients reduced the voluntary intake of food by mouth.

The chief conclusion to be drawn at present from the forced-feeding tests is that the intake of food does not depend upon how much has passed through the mouth. Introduction through a stomach tube or by intraperitoneal injection inhibits a corresponding amount of voluntary ingestion. Plethora inhibits eating for many hours, even when the alimentary tract has not been concerned in the creation of the plethora.

Privation of food. Before the question can be answered as to what guides the rat's intake of food, the sequelae of food privation must be ascertained. Two kinds of privation were imposed on rats, partial or entire. When half as much food was allowed for one day as the 10 rats ordinarily ate ad libitum, the subsequent daily food intake was within 5% of the usual (fig. 9). The same was







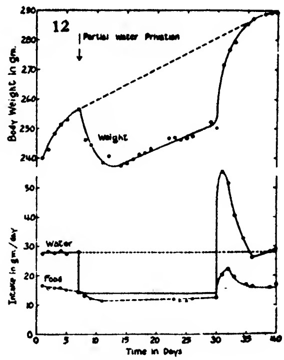


FIG. 9. Food intake was limited to half the voluntary intake in 10 rats for 1 day, and in 2 rats for 2 days. Then both food (F) and water (W) were allowed ad libitum. Mean intakes and standard errors are indicated at the end of each 24-hour period.

FIG. 10. Body weights and ad libitum intakes of food (dash lines) and water (solid lines) in rats deprived of all food and water for 1, 2, 4 or 6 days.

FIG. 11. Daily exchanges of total substance (solids and water) in 4 days of total privation of both food and water and 3 days of recovery upon a regime of unlimited food and water. Mean of 7 rats.

FIG. 12. Body weights and daily intakes in 3 rats which, starting on day 7, for 23 days were allowed half their initial daily intakes of water. Straight broken lines indicate expected values that might have intervened if water intake had not been restricted.

true after food, but not water, was entirely withdrawn for one day (11 rats). Hence there was no indication that the food deficit created on one day was compensated subsequently. Actually the body weight was regained in both experiments without extra intake, solely by the expedient of smaller expenditures. In 2 rats (fig. 9) the partial food limitation was prolonged to 2 days; again the subsequent food intake was the same as before privation, yet body weight was fully

regained. Prolongation of complete food privation to 6 days made no difference in subsequent intake; after prolongation to 7 days the rats failed to eat much and died from delayed effects of the inanition.

Limitation of food intake that was voluntary, as a result of low proportion of nutrients in the cellulose-rich mixtures described above, also resulted in little extra food intake when concentrated food was subsequently made available. Evidently the marked deficits of nutrient intakes consistently aroused no compensatory intakes of significant extra amounts of straight food.

Rather similar results prevailed when water as well as food was denied (fig. 10), but differing in certain details. After this total privation for only one day, 40% extra food was taken as compared with the control period. No greater excess of food was taken after total privation of any longer duration. The intake of 40% extra food differs by a significant amount from the intake after one day's privation of food only, as indicated in the previous paragraph.

From the changes of body weight each day and from the measured intakes, one can compute the total losses (fig. 11). During 4 days of total privation, the losses were greatly reduced. By continuing the reduced rates of loss for one subsequent day upon which full intake was resumed, rats secured nearly all the recovery from inanition that ever occurred. A food deficit was never paid off by subsequent over-consumption.

We conclude that previous privation of food is little or no stimulus to eating. If we call the rat's state one of hunger, then over a 24-hour period hunger does not much augment the animal's intake of food. Though the body be deficient in weight, that weight is restored only slowly through the device of utilizing new food more economically.

Another aspect of this economy was illustrated by Forbes *et al.* (11) who measured the oxygen consumption of rats that were ingesting food at 0, $\frac{1}{2}$, $\frac{3}{4}$, and 1 of their usual voluntary intake. Those on half food almost succeeded in maintaining themselves in energy, for they utilized a larger percentage of the food eaten and expended only 27% more energy than they ate. Those on $\frac{3}{4}$ food expended only 2% more energy than they ingested. Those on full diet used most of the additional utilizable food in extra combustions. Hence a rat has a large margin of dispensable metabolism below what it ordinarily employs upon an ad libitum diet.

Great generalizations cannot be made about the response among species to previous privation of food. In caged dogs the intake was augmented during recovery so that little or no deficiency of intake remained (12, p. 324). Instead the food debt was fully paid off. In rabbits an intermediate situation prevailed (13); in recovery they resembled rats more than dogs.

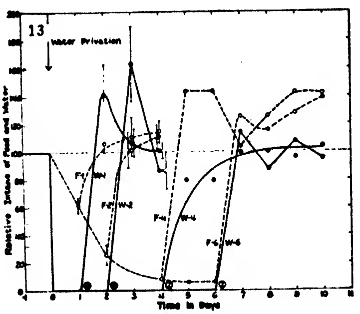
Water availability. When water was denied rats, the amount of food ingested was greatly reduced. The denial was either partial or total.

In one experiment 3 rats were kept for 23 days upon half of their original ad libitum water intake (fig. 12). A new and lower rate of food intake was then set by them, amounting to 75% of the original. Body weight was lost in the first week, but thereafter was slowly regained. Upon restoration of water in-

take ad libitum, the food intake as well as the water intake was markedly increased for 5 days, the food ingestion upon the second day attaining 143% of the original ad libitum rate. But whereas 88 grams of food were missed by each rat during the period of limitation, only 10 grams extra were eaten subsequently, which was less than one control day's intake. Similarly only 23% of the missed water was taken in excess during the recovery. After the water limitation the rats approached but did not attain the meanwhile augmented body weights of control individuals.

More severe self-denial of food was reported by Asher and Hodes (14) in young rats given only one-fourth or one-eight of the usual water intake. Their results and ours are in accord.

When entirely deprived of water, the food intake diminished progressively (fig. 13). After the third day it was less than one-tenth of the original. This



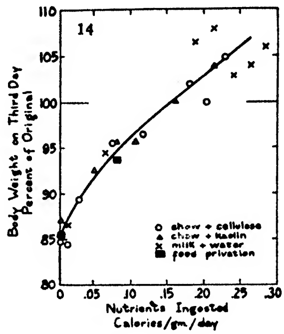


FIG. 13. Ad libitum intakes of food (dash lines) and water (solid lines) in rats deprived of all water for 1, 2, 4 or 6 days.

FIG. 14. Relation of change in body weight to intake of nutrients by rats. The data are derived from figure 2. Intake was limited by direct partial denial of food in only one of the four series. Water was always available ad libitum.

self-denial of food effectively conserved body water, of course. When water was again furnished, only a small excess of water was ingested. This over-all limitation of water ingestion is comparable to the voluntary dehydration that we have described in man. By it the rat economizes in its water requirement. In contrast, a dog economizes very little, since it subsequently drinks most of what it has missed (15); and a man does not economize appreciably in his over-all consumption. To some extent these species differences are related to food intakes during water privation and to body sizes.

When water was given to rats after water privation, moderate excesses of food were eaten; but few exceeded by 40% their original food intakes, and none compensated for all the food self-denied. Water privation thus led to reduced intake of food. This food reduction may be responsible for the moderate amounts of overeating in the recovery period when water was again available in unlimited quantities. Yet, curiously, water privation led to subsequent overeating with

much greater certainty than did food privation per se. It is particularly difficult to emphasize any one accompanying difference, since privation of either food or water led to self-denial of the other. Partial privation of food led to no overeating during recovery, yet the partial self-denial of food that accompanied total privation of water did.

Self-denial of food in proportion to the allowed water intake was reciprocated when limitation of food intake was imposed. Both situations illustrate the tendency of intakes to be relative to one another. The animal's urges to eat and to drink result in an approximate preservation of proportions in its bodily contents. Only when intakes are not limited by the experimenter does inherent volume control express itself. The tendency to proportionality among intakes is also widely found when other constituents of the diet, such as an amino acid or a vitamin, are unavailable; the consumption of all other food items is proportionately self-denied.

Excesses of water intake led to no extra intake of food (fig. 7), even in mixtures of the two in which it would be easy for the rat to ingest extra nutrient in the form of the mixture. Numerous further experiments were done in which water was forced upon the rats, either by stomach tube or by intraperitoneal injection. In those experiments the intake of food over periods of 24 hours was not modified. Of course, in them the excessive water was eliminated by diuresis lasting about 4 hours, leaving 20 hours of the period free of its influence. In contrast to the forcing of food by stomach tube, which inhibited eating for many hours, the forcing of water by stomach tube did not interrupt the periodic ingestion of food.

Comment. Food acceptance and the urge to eat in rats are found to have relatively little to do with a 'local condition of the gastrointestinal canal,' little to do with the 'organs of taste,' and very much to do with quantitative deficiencies of currently metabolized materials. It would be satisfying to know how these deficiencies act in the neuromuscular system that carries out the ingestion. At present there is no sure knowledge of particular sensory areas or afferent pathways. It may be remembered that all kinds of animals have urges to eat, but few have any one pattern of structures.

Evidently the urges to eat form a system of reciprocal relationships among many functions of tissues and organism. Each urge is a resultant of factors whose study, one at a time, is but the beginning of an adequate description. Yet the urges are sufficiently fixed so that the variability of ordinary food intake ($\pm$ 10% among daily periods) is one of the smallest of physiological variabilities, and so that every rat regularly maintains its body weight or increases it slightly each day. Further, the rat returns to its standard intake after each denial of food. The facility and precision with which the organism balances its intake and its output are widely assumed; the assumption needs analytical investigation.

No one characteristic of food guides the rat's intake of it. The investigator gains the feeling that some complex of internal compositions intermittently drives the animal to eat so that some resultant concentration or stimulus is kept just above or below a threshold value. The intake of nutrients may be decreased

by modifying circumstances, but the intake of non-nutrients alone can be increased by changing the composition of the food.

The experiments show how an animal solves several dilemmas that affect the maintenance of its body. In acquiring food, the rat was forced to accept too much roughage or too much water. According to the pattern that was found in these experiments, the rat accepted too much water with little reduction in its caloric intake, until only 2% of its intake was solids and 98% of its intake was water. Thereafter, the weak mixture appeared to furnish diminishing returns from its intake.

When cellulose or kaolin diluted the food, the dilemma was solved by a compromise of a different order. The animal ingested more bulk but stopped before it had ingested a full quota of nutrients. The limited ability to handle roughage in the alimentary tract then became a factor in the animal's urges to eat.

The daily increment of body weight was uniformly related to the potential energy of the food ingested, whether the ingestion was limited by partial privation of nutrients of by offering ad libitum the prepared mixtures of food with cellulose, kaolin or water (fig. 14). The several compromises among physiological components can be expressed as ratios of the excessive intake to the deficient bodily constitution; but none of the other factors studied here actually yield constant ratios.

Ordinarily the urge to eat appears to be governed to a large extent in accordance with the potential energy of adequate food. This fact was shown in the comparison of two concentrated foods of unequal bulk, and in the inhibition of eating when the food had been introduced by a route other than the mouth. But when the animal had been deprived of food either partially or entirely, almost nothing was done to pay back the deficit of food that had been contracted. In the rat (but not in some other species), no prolonged urge to eat resulted from the previous privation. A new sort of adjustment was then manifested, an adjustment between intake and output. Body weight was largely recovered by diminishing the outputs of substance instead of by increasing the intakes. No one had guessed that this dilemma would be solved by such a method. Physical activities were correspondingly reduced, conserving energy.

The ingestion of water that is forced upon the rat by mixing milk with water furnishes a simple technique for securing steady ingestion and excretion. Antidiuretics may be assayed in such rats. Correspondingly, antiposics (agents that inhibit water-drinking) may be identified and assayed in such animals.

The water intake was greatly modified by food intake. Intake of dry nutrients was reduced by two procedures, by diluting the nutrients with kaolin and by limitation of amount of food. In both, the ad libitum intake of water was correspondingly reduced; it bore approximately the same quantitative relation to potential energy of the food that was found in the rats of Strominger (16, fig. 3) which were given artificially limited allowances of food.

The rat would ingest much extra water in obtaining its daily quota of food; but it did not ingest excesses of dry food as a means of obtaining the water that was in it or potentially obtainable by oxidation of it. The physiologist knows

that excess of food would dehydrate the rat still further, for water is extracted from tissues by the metabolic products of the food that become available for excretion. The rat without that knowledge still has a pattern of appetites that seems appropriate in the light of the knowledge.

Most dietary experiments imply the assumption that the animal consumes as much of the available food mixture as will improve the animal's nutritive status. Trust is placed in paired feeding as the sole control procedure. The experimenter has no information as to whether or not forced feeding would change the status in one direction or another. The urges to eat must, therefore, be distinguished from the abilities to utilize the nutrients; each requires its own investigation, as is well recognized in studies of human food acceptance.

It seems evident that the regulation of food intake is under many influences. While it receives precedence over many activities of the rat, yet it is also inhibited by some. While intake looks like a simple problem in alimentation when the animal is kept in a sufficiently simplified situation, the animal has a full pattern of priorities and compromises that also solves situations of great complexity. All animals that have been studied, those without alimentary tracts as well as those which have, recognize food, spurn food when it is superabundant, and put forth extra efforts to get it when it is rare. Hence, whatever be the machinery that may fix the pattern of priorities in rats, comparable patterns seem to be endowments of all animals, whether or not they possess specialized neuromuscular or alimentary systems.

SUMMARY

1. Albino rats were furnished food that was mixed with some proportion of cellulose, kaolin or water. In most mixtures the total bulk eaten exceeded considerably the bulk of concentrated food consumed in control periods. The quantity of utilizable nutrients ingested did not exceed that in control periods.

2. In this manner rats were regularly induced to ingest roughages to the mean extent of 8% of their body weights per day, and water up to 125% per day. In the presence of roughages, a compromise was effected between an excessive amount of alimentary fill and a diminished amount of nutrients that depended upon the mixture available. In the presence of water, the upper limit to ingestion was probably not fixed by the maximal rate at which water could be excreted through the kidneys.

3. A variety of flavorings and nutrients in small concentrations did not induce rats to consume greater amounts of roughages.

4. The relations between food intake and water intake were also investigated by limiting the available amounts of one or the other. The intake of the unlimited item (food or water) was regularly reduced but not so greatly as that of the limited item.

5. After complete privation either of food or of water or of both for 1 to 6 days, body weight was subsequently slowly restored with the consumption of very small excesses. Deficits of intake were not paid off; instead, the outputs were reduced for a day or two. Recovery itself was also compromised in such a

manner that animals temporarily deprived did not catch up to the body weights of those not deprived.

6. Patterns of ingestion and excretion were manifested that showed the coordinations among the several factors of turnover. Not only was water ingestion tempered to excretory capacities, but also water ingestion to food ingestion, and food ingestion to absorptive and roughage-handling capacities. These patterns are items in a large complex of regulatory activities concerned in bodily maintenance.

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EFFECT OF ESTROGENS ON THE BODY AND ORGAN WEIGHTS AND THE ARGINASE AND 'ALKALINE' AND 'ACID' PHOSPHATASES OF THE LIVER AND KIDNEY OF CASTRATED MALE MICE[1]

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Received for publication August 2, 1947

In previous reports the effect of various steroids on the organ weights (1, 2) the arginase activity (3, 4) and the 'alkaline' and 'acid' phosphatases (5, 6) of the kidney of the mouse have been reported. These studies now have been extended to the available naturally occurring estrogens.

Procedure. Male mice were castrated at 16.5 to 19.0 grams body weight; one month later pellets of the various estrogens[2] were implanted subcutaneously (1, 2). Mice of the Murray-Little dba[3] strain were used for the studies at the higher dose level but they were not available for the experiments at the lower dose. The Swiss strain of mice from our bacteriology department's colony was found to respond to steroid stimulation in a manner similar to that of the dba strain. Therefore, they were used to complete this project.

The food, Rockland Rat diet, was removed from the animal cages on the 29th day after implantation of the pellets and the mice were killed by decapitation on the 30th day. The organs were removed and weighed on a Roller-Smith torsion balance. A 2-mm. section was cut transversely through the middle of one of the kidneys and placed in cold redistilled acetone for the histochemical determination[4] of the 'alkaline' phosphatase (7). The remainder of the kidneys and the liver were homogenized, and the arginase and the 'alkaline' and 'acid' phosphatase activities were determined as previously described (3–6).

Results. *Rate of absorption of the estrogens.* The most soluble estrogen was the benzoate of estradiol. The pellet of this material lost its cohesiveness and at recovery crumbled at the slightest pressure. In one instance (cf. table 1), the pellet had crumbled during the course of the experiment and the material was completely absorbed. The dipropionate of α-estradiol, on the other hand, maintained its cohesiveness and was only slightly less soluble than the unesterified estrogen. Estriol was as soluble as α-estradiol dipropionate. Equiline and estrone were approximately equally soluble at a rate slightly less than that of estriol.

Mixture of the estrogens with cholesterol in a ratio of 1:3 decreased the rate of absorption 300- to 400-fold.

[1] This investigation was aided by the Josiah Macy, Jr., Foundation.

[2] The estriol was provided by Dr. D. McGinty of Parke, Davis and Co. and the other estrogens by Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc.

[3] These mice were provided by Dr. S. G. Warner of the Biological Station, Springville, New York.

[4] Charles Luttrell assisted in these determinations.

Body weight. The mice implanted with pellets of pure estrogens gained less than their controls and in most instances lost weight. The losses probably were even greater than indicated (table 1) because the urinary bladders of these mice were found at autopsy to be distended with urine. The one mouse whose pellet of estradiol benzoate was crushed during the course of the experiment showed the most marked decrease in body weight.

TABLE 1. *The effect of estrogens on the body and organ weights of castrated male mice*

TREATMENT	MICE	PELLETS[1]	ESTROGEN ABSORBED	BODY WEIGHT		% CHANGE FROM CONTROLS		
				Grams	Change[2]	Sem. ves. + pros.	Kidneys	Thymus
Control (dba)	5	(1)	—	23.7	1.2	(9)[3]	(284)	(35)
Control (Swiss)	11	(1+3)	—	23.5	0.2	(9)	(228)	(43)
Estrone	4	1	1.05	21.7	0.0	+167	+2	−69
Estrone	5	1+3	0.03	26.1	2.3	+278	+13	−60
Equiline	4	1	1.20	21.7	−0.3	+156	−4	−52
Equiline	5	1+3	0.05	25.0	2.6	+222	+12	−60
Estriol	4	1	1.90	19.9	−2.0	+144	−14	−77
Estriol	5	1+3	0.07	26.6	2.3	+267	+9	−58
α-Estradiol[4]	6	1	2.6	20.0	0.1	+54	+15	−70
α-Estradiol[4]	5	1+3	0.06	23.3	2.1	+278	+16	−75
α-Estradiol benzoate	3	1	6.4	20.3	−2.0	+89	+4	−83
α-Estradiol benzoate	1	1[5]	13.2	17.0	−4.3	+78	−24	−99
α-Estradiol dipropionate	4	1	1.9	20.1	−0.7	+122	−3	−77
α-Estradiol dipropionate								

[1] The 1+3 designation in this column indicates the estrogen: cholesterol composition of the pellet.
[2] The change in grams body weight during the 30-day experimental period.
[3] The figures in parentheses are in milligrams.
[4] These values are taken from a previous report (1).
[5] The pellet of this mouse crumbled during the experiment and was completely absorbed.

The mice which received the lower dose of estrogens all gained in body weight and did not show any unusual distention of the urinary bladder at autopsy except those mice which received the α-estradiol.

Organ weights. The seminal vesicles and prostates were increased somewhat more by the very small than the large dose of the estrogens. The kidneys were not notably increased in weight by any of the estrogens. There were evidences of small increases after treatment with α-estradiol at both dose levels. The other estrogens, however, had no effect at either the high- or low-dose levels. There was, moreover, a marked decrease in the kidney size of the mouse which received the large dose of α-estradiol benzoate. This is probably a reflection of the poor nutritive state of the animal (cf. table 2) as a result of the excessive

dosage of the estrogen. The thymus was markedly decreased by all of the estrogens. The lower dose was as effective as the higher dose.

Enzymes. The enzyme activities of the tissues of both the dba and the Swiss white mice controls were approximately the same (table 2).

All of the estrogens produced a marked but not very great increase in the arginase activity of the kidney. This increase was roughly the same for all of the estrogens. The higher dose of estrogen did not produce a further increase. Indeed, it may have produced a smaller response, e.g., equiline. All of the estrogens except α-estradiol produced a small but questionable increase in the arginase activity of the liver.

TABLE 2. *The effect of estrogens on the arginase activity of the kidney and liver of castrated male mice*

TREATMENT	MICE	ESTROGEN ABSORBED	KIDNEY		LIVER	
			Mgm.	Arginase	Mgm.	Arginase
				%[1]		%[1]
Control (dba)	4	—	284	(42)[2]	980	(16500)[2]
Control (Swiss)	11	—	228	(48)[2]	840	(15900)[2]
Estrone	4	1.05	291	+64	1000	+20
Estrone	5	0.03	265	+63	1100	+30
Equiline	4	1.20	272	+38	972	+6
Equiline	5	0.05	263	+104	1070	+31
Estriol	4	1.90	262	+45	1101	+12
Estriol	5	0.07	254	+69	1198	+36
α-Estradiol	6	2.6	316	+88	—	—
α-Estradiol	5	0.06	258	+100	921	+18
α-Estradiol benzoate	3	6.4	295	+67	1136	+40
α-Estradiol benzoate	1	13.2	215	+222	776	+38
α-Estradiol dipropionate	4	1.9	275	+69	1017	+43

[1] Per cent change from the respective castrated controls. The dba mice were used at the high-dose and the Swiss mice at the low-dose levels.

[2] These values are in units per gram.

The 'alkaline' and 'acid' phosphatases of both the liver and kidney were not altered after estrogen administration. The differences were never greater than plus or minus 15% from that of the respective control values. Furthermore, the histochemical study of the 'alkaline' phosphatase of the kidney did not reveal anything worthy of note.

DISCUSSION. It is of interest that esterification of estradiol does not produce a marked decrease in the rate of absorption of this material since esters of the androgens are much less soluble (8, 1). Indeed, the benzoylation of α-estradiol increased the solubility. It is of further interest that in contrast to the androgens the diol of the estrogens, α-estradiol, is more soluble than the 3-hydroxy-17-

ketone compound, estrone. These differences between the two types of steroids are due probably to the different nature of the *A* ring of the molecules.

The effect of the high dosage of the estrogens on the body weight and urinary bladder was expected. These effects have been noted by many investigators. The effect of the estrogens on the seminal vesicles and prostates also is well known. It is of interest that the estrogens were equally effective at the high- and low-dose levels. The appearance and 'feel' of the seminal vesicles indicated that the growth was not similar to that observed after androgen treatment. It is noteworthy that the amount of estrogen necessary to bring about this change is extremely small and that it occurs even though the animal is losing considerable body weight as a result of over dosage with subsequent undernutrition. The kidneys, in contrast to the accessory sex organs, not only are not stimulated to increase in size by the estrogens but actually decrease when the dose of the estrogen is such as to cause a decrease in body weight (cf. Table 2).

It is remarkable that the arginase of the kidney increases without any comparable increase in kidney size. In the case of the androgens the enzyme is not increased until the kidney size has been restored to and above normal. Furthermore, an increase in the amount of estrogen administered does not produce a further increase in arginase activity as in the case with the androgens (4). The persistent but small increase in the arginase activity of the liver is not remarkable (cf. table 2).

The failure to find any marked changes in the phosphatases of the kidney indicates that these enzymes are not associated with the well-known formation of kidney stones after estrogen overdosage.

SUMMARY

Male mice castrated at 16.0 to 19.5 grams body weight were implanted subcutaneously with a pellet of pure estrone, equiline, estriol, α-estradiol, α-estradiol benzoate and α-estradiol dipropionate. The first four estrogens also were implanted as pellets consisting of one part of estrogen and three parts of cholesterol. After 30 days the mice were autopsied. The addition of cholesterol to the estrogens decreased the rate of absorption about 300- to 400-fold. The estrogens increased the body weight at the lower dose but inhibited or decreased it at the higher dose. The kidneys were not or only slightly increased in size. The thymus was decreased and the seminal vesicles and prostates were increased about the same by both doses of estrogens. The arginase activity of the kidney was increased equally by both dose levels. The arginase activity of the liver was not remarkably increased.

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THIOURACIL, BASAL METABOLISM, AND SPECIFIC DYNAMIC ACTION

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Received for publication August 25, 1947

It is generally known that some plants, such as cabbage (1), soybeans (2) and Brassica or rape seeds (3), contain substances which produce hypothyroidism by blocking the formation of thyroxine. The thyroid then becomes hyperplastic because of the increased pituitary secretion of thyrotropic hormone (4).

Recently several synthetic goitrogens, especially thiourea and thiouracil, attracted wide attention because of their potentialities in medicine for depressing overactive thyroids (5) and in agriculture for reducing the maintenance cost and thereby increasing the economy of the last stages of fattening (6). Several categories of goitrogens are discussed in the literature, including cyanides (in cabbage and soybeans); thiourea derivatives (in Brassica or rape seeds); sulfonilamides (7); as well as azides, sulfides, carbon monoxide, arsenic etc., which interfere with oxidoreduction systems, such as the cytochrome cytochrome-oxidase system. This suggested the theory (8) that some goitrogens are antioxygenic, perhaps interfering with the oxidation of iodide ion to elementary iodine, the form in which it apparently enters the thyroxine (9). Astwood (10) postulated that derivatives of thiourea, aminobenzene and sulfonamides possess similar goitrogenic properties because of their structural similarity to tyrosine, which therefore compete, in vitamin anti-vitamin fashion, with diiodotyrosine for the enzyme system concerned in its conversion to thyroxine.

Thiouracil and basal metabolism. Thiouracil is currently the most popular thyroid-depressing drug because, unlike thiourea, for example, it is destroyed by most body tissues (except bone marrow) and is therefore less toxic (11). Since the metabolic rate is the best index of thyroid activity, it was thought instructive to map the time relations of its decline by measuring the rate of oxygen consumption following the beginning of feeding thiouracil and its rise following the cessation of thiouracil feeding.

The data were obtained on 150- to 200-gram male rats at 26°C. fed a stock diet containing 0.1% thiouracil. Adding thiouracil to the feed reduced its consumption by about 10% of the normal level, so that each animal consumed 22 to 25 milligrams thiouracil per day in contrast to the customary daily dosage of 0.6 grams for man. The oxygen consumption was measured in an 8-chamber Regnault-Reiset-Kleiber type of apparatus (12).

The oxygen consumption decreased to 60% of the normal level by the 17th day of thiouracil feeding, and returned to the original, normal, level 18 days (in contrast to about 35 days in man) after stopping its feeding (fig. 1). The effect

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of feeding thiouracil at the given level on the basal metabolism is evidently similar to that of surgical thyroidectomy.

Thiouracil and specific dynamic action (SDA). As thyroxine is perhaps the most powerful metabolic accelerator in the body, considerable attention has been paid to its influence on SDA but with indifferent and contradictory results (13).

A comparison was, therefore, made of the SDA of three amino acids when fed to normal and to thiouracil-fed rats. The amino acids were administered as sodium salts by stomach tube after the rats fasted about 12 hours. The resulting

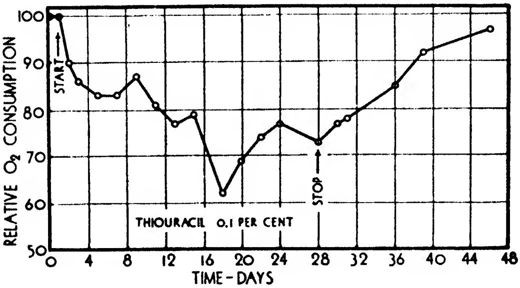

FIG. 1. Time course of metabolic decline in rats on a diet containing 0.1% thiouracil, and metabolic recovery following cessation of feeding thiouracil. Each rat consumed 22 to 25 mgm. thiouracil per day.

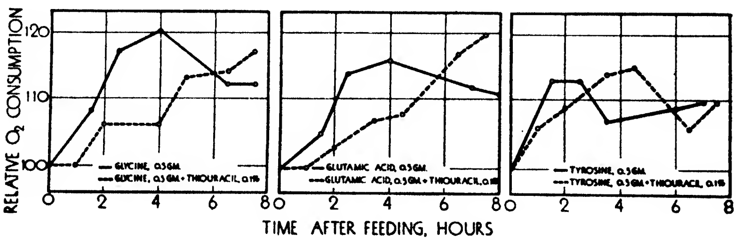

FIG. 2. Relative effects of thiouracil on the specific dynamic action of glycine, glutamic acid and tyrosine administered after a 12-hour fast.

data are shown in figure 2; each point represents the average of 8 male rats, 150 to 200 grams in weight.

The metabolic level in the thiouracil-fed rats is seen, in figure 2, to be relatively depressed and the time relations delayed for glycine and glutamic acid. This delay may be due to three causes: thiouracil was observed by some investigators (personal communication) to damage the gastrointestinal mucosa and to delay absorption; thyroidectomy is known to reduce the absorption rate of carbohydrate and, therefore, perhaps of amino acids; protein tends to be stored under the skin in hypothyroid animals, and stored protein is without SDA.

The SDA of glycine and glutamic acid was definitely delayed and depressed by thiouracil feeding, but not of tyrosine (right curve in fig. 2), at least not to a

statistically significant extent (15). This may, perhaps, be explained by assuming that the SDA of tyrosine is so low in normal animals that thiouracil feeding cannot lower it further; and that the SDA of tyrosine is low in normal animals because of its chemical similarity to thyroxine with which it, therefore, probably competes and tends to displace, with consequent reduction of heat production (SDA). This explanation of displacement of thyroxine by tyrosine is substantiated by Woolley's report (14) that the metabolic effect of thyroxine can be reduced by administering tyrosine derivatives.

To test this hypothesis of competition between tyrosine and throxine, rats were fed diets containing 0.1% thiouracil for thyroxine depletion. They were then injected with 5 mcg. thyroxine per 100-gram rat weight, and half of these animals were fed a diet to which was added 10% tyrosine. The thyroids of the tyrosine-fed rats were 12% heavier than those not receiving the tyrosine, and this difference in thyroid weight is statistically significant (15), indicating that tyrosine, like thiouracil, enlarges the thyroid gland or induces hypothyroidism probably because tyrosine competes with thyroxine in the tissues.

The above anatomical observation that tyrosine feeding enlarges the thyroid was substantiated metabolically by feeding rats 10% tyrosine and injecting them with 100 mcg. thyroxine per 100-gram body weight and measuring the metabolism. The metabolism (during a 7-day period) increased to a smaller extent in the tyrosine-fed than in the control animals (15) probably because the tyrosine neutralized or displaced some of the thyroxine.

SUMMARY

1. A chart is presented showing the daily decline in the metabolic rate (oxygen consumption) of rats with time of feeding thiouracil and the recovery therefrom after cessation of thiouracil feeding. The lowest metabolic level of 40% below the normal was attained 17 days after placement on the diet. On removing the thiouracil from the diet the metabolic rate returned to normal in 18 days.

2. Rats on the thiouracil diet showed a depressed and delayed specific dynamic action for glycine and glutamic acid, but not for tyrosine, thus substantiating the theory that tyrosine competes with and tends to displace thyroxine from the active centers of tissue cells.

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RELAXATION OF THE PELVIC LIGAMENTS IN GUINEA-PIGS BY ESTROGENS AND PROSTIGMINE*

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Received for publication July 19, 1947

The long gestation period of guinea-pigs allows for unusual intrauterine growth of the embryos and at full term the young are much larger than the pelvic outlet. In order that birth may occur the bones of the pubic symphysis separate a half-inch or more at the time of parturition (1). That this relaxation of the pelvic ligaments is the result of hormone control was first shown by the use of pregnant rabbit serum (2); later a more specific substance was obtained by Hisaw to which he gave the name of relaxin (3) and, more recently, the details of the properties of relaxin and a review of the later literature were published (4).

Since progesterone will relax the pubic ligaments of guinea-pigs it was of interest to find that desoxycorticosterone acetate acted in this respect like progesterone (5). In the process of collecting these data it was observed that some of the guinea-pigs showed very little relaxation of the pelvis while others given identical treatment showed marked pelvic relaxation. This led to an attempt to increase the extent of pelvic relaxation by the use of a vasodilator substance. The prostigmine test for pregnancy (6) always seemed to be a bizarre reaction, and since the uterus may be involved in relaxation of the pubic ligaments (4) it was decided to try to find out more about prostigmine by studies on the pelvis of guinea-pigs. To our surprise it was found that within a day or two after prostigmine was injected the pelvis of the guinea-pig could be shaken more freely than ever observed when relaxation was induced by progesterone.

METHODS. These preliminary observations led to the present study. A total of 48 guinea-pigs were used and in many of them repeated tests were made. They were all young animals and some of them weighed as little as 250 grams. Bilateral oöphorectomy preceded the tests unless otherwise stated. The estrogens[1] were given subcutaneously (theelin in aqueous suspension and stilbestrol in oil) for a period of one to a few days and over a range of doses. A few days later prostigmine[2] was given subcutaneously twice daily in the amount of 0.05 mgm. in 0.5 cc. of saline solution. Usually prostigmine was not continued longer than one day, but in some cases it was repeated several days in succession. The pelvis was examined manually as previously described (5) and the degree of relaxation estimated at 8, 18, and 24 hours, and daily thereafter for several days. X-ray examination was also employed and in some animals fluoroscopic views were observed.

* Research paper No. 845, Journal Series, University of Arkansas.

[1] The estrogens in the form of theelin and stilbestrol were generously supplied by Dr. O. Kamm, Parke Davis & Co., and Dr. Erwin Schwenk, Schering Corporation.

[2] Prostigmine was kindly supplied by Dr. E. L. Sevringhaus, Hoffman-LaRoche, Inc.

Observations and results. In experiments of this kind, where the measuring stick is the operator's judgment of pelvic relaxation as determined by palpation, the symbols used to express the results are at best only estimates. Never-

1

2

3

4

Fig. 1. Theelin, 0.5 mgm., injected daily for 4 days; rest period 2 days; prostigmine, 0.1 mgm., given at one injection. X-ray taken 7 hours later. Fig. 2. Stilbestrol, 0.1 mgm., injected daily for 4 days; prostigmine, 0.1 mgm., injected daily for 2 days. X-ray taken 3 days later and at maximum relaxation. Fig. 3. Theelin, 0.5 mgm., injected daily for 4 days. X-ray taken on 5th day. No pelvic relaxation. The separation between the pubic bones was filled solidly with fibrous tissue. Fig. 4. Guinea-pig as shown in fig. 3 continued; rest period on 5th and 6th days. Prostigmine, 0.1 mgm., on 6th day. X-ray on 8th day.

We are indebted to the Staff of the X-ray Department for the roentgenograms.

theless it seemed of some value to list our readings from 1 to 4+, and the data are so arranged in table 1. X-ray studies have confirmed these results (fig. 1–4).

It will be noted from table 1 that estrogens were given in a wide range of dos-

age. This seems to be unnecessary since guinea-pigs receiving small amounts (0.5 mgm. or less) apparently had the pelvic ligaments properly conditioned for the action of prostigmine. It seems likely that daily injections of estrogens are more important than a single injection of a larger amount. We have also confirmed that pelvic relaxation may be induced by estrogens alone if treatment is continued for a period of about 20 days (7 and citations by 4).

Prostigmine in amounts of 0.1 mgm. given as a single injection killed some of the guinea-pigs and in two cases 0.05 mgm. was fatal. Relaxation of the pelvis can be induced by a single injection of 0.05 mgm. but more consistent results were obtained when a second injection followed the first by a few hours giving a total of 0.1 mgm. of prostigmine. Relaxation may occur within 8 hours (fig. 1) if the pelvis is already slightly loose or may be delayed for as long as 4 or 5 days.

The degree of relaxation varied, as shown in table 1, but as a whole the relaxation with prostigmine was more marked and more consistent than had been

TABLE 1. *Degree of relaxation in the pelvic ligaments, shown over an estimated range from negative to 4+. Figures in parentheses are the numbers of cases observed. T—theelin; S—stilbestrol*

NO. OF TESTS	ESTROGEN	PROSTIGMINE	DEGREE OF RELAXATION IN PELVIC LIGAMENTS
	mgm.	*mgm.*	
10	0.5 T	0.1	(1) −, (1) +, (2) ++, (3) +++, (3) ++++
28	1 to 2 T	0.1	(2) +, (9) ++, (5) +++, (12) ++++
11	2 S	0.1	(3) ++, (3) +++, (5) ++++
20	None	0.2	(18) −, (2) +
		Not Castrated	
22	None	0.2	(5) −, (9) +, (8) ++
10	2 T	0.1	(2) ++, (6) +++, (2) ++++

obtained with progesterone or desoxycorticosterone acetate (5). However, we have not yet devised a procedure that will produce maximum pelvic relaxation in all guinea-pigs.

Prostigmine given alone will relax the pelvis if the effects of estrone are still evident. In our abstract (8) reference was made to this effect of prostigmine. Subsequently we have tried giving prostigmine to a group of young females a few days after they were oöphorectomized and to chronic castrates without estrogens and they did not show relaxation of the pelvis. Twenty observations are shown in table 1. Thus it seems evident that relaxation of the pelvis induced by prostigmine must be preceded by a conditioning of the structures involved; in these experiments this conditioning was effected by the action of either theelin or stilbestrol. Perhaps the guinea-pig is not unlike the rat, in that a single large dose of estrone may give an estogenic response for 2 or 3 weeks following the injection (9). During this time interval the pelvis of the guinea-pig is responsive to prostigmine.

In uncastrated virgins (table 1) prostigmine gave almost no effect. These

observations may indicate that the ovaries protect the pelvis from the action of prostigmine. However, when such guinea-pigs are primed with theelin relaxation of the pelvis is quite readily induced by prostigmine and the degree of relaxation is of about the same magnitude as that obtained by the same procedure after oöphorectomy. Therefore it would seem that the virgin guinea-pig has too little estrogen in the circulation to condition the pelvis for the action of prostigmine.

Six guinea-pigs were oöphorectomized and hysterectomized and then injected with theelin and prostigmine. Pelvic relaxation was obtained.

The reports of relaxin (4, 10, 11, 12) seem to place it as the normal relaxing agent for the pelvis in pregnancy and perhaps even playing a rôle in estrus although it is not estrogenic (11). We have no evidence that a prostigmine-like substance, cholinesterase or acetylcholine play a rôle in pregnancy.

Acetylcholine was given to several guinea-pigs as a substitute for prostigmine and although some evidence of relaxation of the pelvis was found the results were poor and usually negative. Therefore the data available do not warrant further consideration of acetylcholine at this time.

SUMMARY

Forty-eight normal and oöphorectomized guinea-pigs were used in studies on experimental relaxation of the pelvic ligaments. Relaxation of the pelvic ligaments was induced by long-continued action of either theelin or stilbestrol. It was found that theelin and stilbestrol conditioned the pelvic ligaments within a few days in such a way that relaxation was readily induced by prostigmine. This conditioning effect lasted for as long as three or four weeks after the last injection of estrogen.

Prostigmine given alone to oöphorectomized young virgin guinea-pigs did not induce relaxation of the pelvis. The ovaries do not seem to protect the pelvis from the action of prostigmine if the pelvic ligaments have been conditioned by the action of estrogens. Pelvic relaxation was obtained after hysterectomy.

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TOLERANCE TO POTASSIUM INTOXICATION IN THE ALBINO RAT

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Received for publication August 9, 1947

The ability of living organisms to develop a tolerance to a wide variety of toxic substances is well known In the course of some studies involving the repeated administration of just sublethal doses of potassium chloride to rats by stomach tube, severe toxic symptoms were produced for the first few days. However, it was noted that subsequent doses of potassium chloride gradually ceased to elicit these symptoms and the animals apparently developed an increased resistance to this treament. Allers, Nilson and Kendall (1) have apparently made similar observations in normal dogs. In view of the great importance of the potassium ion in physiologic processes, the nature of this increased resistance to potassium intoxication seemed to warrant further investigation. This study demonstrates that a true systemic tolerance to the potassium ion can be developed in rats. Some of the possible contributing factors to such a tolerance have also been investigated.

METHODS. A total of 625 albino rats weighing between 160 and 220 grams were used in these experiments. Male rats were used exclusively, except for the 40 female rats which served to determine whether there was a sex difference involved in the development of a tolerance or in the natural resistance to the potassium ion. A group of control animals was always tested simultaneously with the experimental animals. However, since the day-to-day variation did not exceed the variation within the different groups, all animals of the same sex and receiving the same type of treatment have been considered as a single group. As a result, the statistical analysis includes the day-to-day as well as the group variation.

All animals were kept on a diet of Purina Dog Chow checkers and tap water. They had access to food and water throughout the experiments. Both control and experimental animals were kept in groups of 5 rats to a cage in a constant-temperature room (78°F.).

A no. 8 rubber catheter attached to a 10 cc. hypodermic syringe was used for administration of the various salt solutions. This was found to be more satisfactory than the rigid tubes sometimes employed.

Test procedure. Preliminary experiments were carried out to determine the frequency of administration and size of dose of the potassium salts required to kill all of the control rats at a rate which would demonstrate a measurable difference between control and experimental animals. The most satisfactory oral test procedure was the administration of 25 cc. of a 1.35 molar solution of the potassium salt per kilogram of body weight, four such doses being administered at four-hour

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intervals. The maximal time allowed for any experiment was 16 hours, i.e., 4 hours after the administration of the fourth dose. The total survival time was taken as a final index of tolerance, and the statistical analysis was applied to this value.

With this test procedure, no control animal lived to receive more than 3 doses of the potassium solution. The length of survival was measured in minutes from the time of administration of the first dose. An animal was not considered dead until the heart had stopped beating.

As would be expected, potassium given intraperitoneally was much more toxic than that given orally and the dosage level was extremely critical. A 0.4 molar solution of potassium chloride was found to be the most satisfactory concentration for the intraperitoneal test. With the exception of this change in concentration of potassium, the procedure for the intraperitoneal test was identical with that for the oral test.

Adaptation procedure. Rats were adapted to the potassium ion by daily administration of 4 doses of 1.35 molar salt solution by stomach tube at 8 A.M., 12 M., 4 P.M. and 8 P.M. An initial sublethal dose of 10 cc. per kilogram of rat was found to be satisfactory. The size of the dose was increased by 5 cc. every 4 days until a dose of 25 cc. of potassium solution per kilogram of rat was reached. The next day the resistance of adapted animals was compared with that of control animals by subjecting both groups to the test procedures previously described. This conditioning regimen did not result in any fatalities, nor did it prevent the rats from gaining weight at the normal rate.

Simplification of the procedure by administering the potassium salt solution 3 times a day at 3-hour intervals and increasing the dose every 3 days resulted in a few fatalities during the conditioning period. The studies of Norris and Elliott (2) on the development of tolerance to arsenic, and of Liling and Gaunt (3) on acquired resistance to water intoxication, suggested that the course of adaptation could be followed by observing changes in body temperature. However, in our experiments, no change in body temperature during the course of adaptation was found.

RESULTS. *Toxicity of potassium salts to normal rats.* The relative toxicity of various potassium salts administered orally in doses containing equal amounts of the potassium ion is shown in table 1. It is to be noted that there was no significant difference in the toxicity of potassium chloride, potassium acetate, and potassium citrate. On the other hand, potassium bicarbonate caused death of the animals in a much shorter period, the difference being highly significant (P = less than 0.01). This might be expected in view of the fact that potassium bicarbonate is an alkalinizing salt, and that K^+ and OH^- are usually synergistic in their physiological reactions (Heilbrunn, 4; Fenn, 5).

Although there appeared to be a difference in the natural resistance of the male and female controls to the test procedure, this difference is not significant, since the P value of Fisher exceeded 0.05.

Studies on adaptation to the potassium ion. Both the male and the female animals (table 1, groups II and IV) adapted to potassium chloride were completely

tolerant to the test procedure. All of these animals survived the 4 test doses of potassium chloride and all were alive 24 hours after the test. As seen in table 1, the mean total survival time of the male control animals (group I) was only 283 minutes, and that of the female controls (group III) was only 258 minutes. None of the control animals survived to receive the fourth test dose of potassium chloride. There was no difference between male and female rats in their development of a tolerance to potassium chloride.

Studies on both male and female rats which had been adapted by administering potassium chloride solution 3 times a day at 3-hour intervals, the dose being increased every 3 days, gave results which were identical with those just described.

TABLE 1. *Toxicity of various potassium salts to 'normal' adapted rats*

GROUP	NO. OF RATS	ADAPTATION (ORAL)	TEST (ORAL)	SURVIVAL									
				1st dose		2nd dose		3rd dose		4th dose		Total	
				Min.[2]	%[4]	Min.[3]	%[4]	Min.[2]	%[4]	Min.[2]	%[4]	Min.[3]	Es[5]
I	115♂	None	1.35M KCl	218	83	62	17	3	0			283	±11.1
III	10♀	None	1.35M KCl	220	90	38	0					258	±25.6
VI	30♂	None	1.35M KAc[1]	236	90	67	13	3	0			306	±16
XXII	20♂	None	1.35M KCit	211	75	96	30	27	0			334	±40
XXIII	25♂	None	1.35M $KHCO_3$	111	80	6	0					117	±15.8
II	73♂	1.35M KCl	1.35M KCl	240	100	240	100	240	100	240	100	960	No variation
IV	10♀	1.35M KCl	1.35M KCl	240	100	240	100	240	100	240	100	960	No variation
V	10♂	1.35M KAc	1.35M KAc	240	100	240	100	240	100	240	100	960	No variation
VII	10♂	1.35M KCl	1.35M KAc	240	100	240	100	240	100	240	100	960	No variation
VIII	10♂	1.35M KAc	1.35M KCl	240	100	240	100	240	100	240	100	960	No variation
XI	15♂	1.35M KCl	1.35M KCl	223	87	146	53	45	13	26	7	431	±60.8
X	19♂	None	0.4M KCl(i.p.[6])	53	10	2	0					55	±17.0
XI	7♂	1.35M KCl	0.4M KCl(i.p.[6])	183	71	21	0					204	±42.6

[1] Potassium acetate.
[2] Mean survival time in minutes following each test dose.
[3] Mean total survival time in minutes.
[4] Per cent of animals surviving four hours after each test dose.
[5] Standard error.
[6] i.p. = intraperitoneal.
Group XI. Tested after receiving no treatment for seven days following adaptation.

In view of the possibility that the tolerance phenomenon considered here might be entirely or in part due to the anion of the salt or to the cation-anion combination employed, studies were carried out in which animals were conditioned and tested with different potassium salts.

A group of animals was adapted with 1.35 molar potassium acetate solution (table 1, group V) and tested with potassium acetate. As was true of the rats conditioned and tested with potassium chloride, all animals received all 4 test doses and all were alive 24 hours after the experment. A second group of animals which had been adapted to potassium chloride was found to be completely tolerant when tested with potassium acetate (table 1, group VII). Likewise, a third group of rats conditioned with potassium acetate demonstrated a complete tolerance when tested with potassium chloride (table 1, group VIII). It is to be recalled that potassium acetate and potassium chloride were equally toxic to normal rats.

Since the potassium ion had been administered by way of the gastro-intestinal tract in the adaptation procedure, the question arose as to whether the results represented a true systemic tolerance or were simply due to a decreased absorption of the salt. In order to test this possibility, rats adapted with potassium chloride by the oral route, as in the previous experiments, were then tested with potassium chloride administered by the intraperitoneal route (table 1, group IX). The tolerance of these animals was found to be highly significant when compared with control animals tested by the intraperitoneal route (table 1, group X) (P less than 0.01).

Additional evidence that the adapted animals had a true systemic tolerance was the absence of any diarrhea, which would be expected if the phenomenon were due simply to decreased absorption of the salt.

Fifteen animals which had been adapted to potassium chloride were tested with potassium chloride after having received no treatment for 7 days (table 1, Group XI). These animals had a total mean survival time of 431 minutes, one of the animals being alive 24 hours after the test. Comparison with male control rats (table 1, group I) shows that a significant degree of tolerance persists for at least 7 days after cessation of treatment (P value of Fisher less than 0.02).

The possible non-specific influences of *a*), passage of the stomach tube, *b*), sudden distention of the stomach with liquid, or *c*), large water shifts caused by administration of hypertonic salt solutions were examined as contributory factors in the adaptation process. Three groups of animals for these experiments were prepared by the treatments shown in table 2 and then tested by the oral route with 1.35 molar potassium chloride solution. Although all 3 groups showed a greater resistance to the test procedure than the male controls (table 1, group I), the difference was of slight significance.

Potassium tolerance of rats adapted to water intoxication. As would be expected, the water consumption increased to a high level during the period of adaptation. The water intake for 24 hours of 16 tolerant rats receiving 4 test doses of potassium chloride was found to be 390 cc. per kilogram and that of 9 tolerant rats receiving 4 test doses of potassium acetate was found to be 322 cc. per kilogram, whereas 50 normal rats consumed only 150 cc. of water per kilogram. The water consumption of tolerant animals returned to normal levels within 24 hours after cessation of treatment.

Nine rats receiving 4, 25 cc. doses of 1.35 molar sodium chloride solution per kilogram, in place of the potassium solution in the adaptation procedure, consumed 427 cc. of water per kilogram in a 24-hour period.

The additional observation was made that after the rats had been subjected to the adaptation procedure for a few days, their urine output increased markedly. That the entire volume of liquid ingested was rapidly excreted by the kidneys was shown by the fact that the adapted animals demonstrated no abnormal weight gain during the test day.

In view of the large volume of water consumed by potassium-tolerant rats, a study was made to determine whether a resistance to the toxic effects of excess water, as reported by Liling and Gaunt (3), would protect rats to any degree against potassium poisoning.

Forty male rats were conditioned by administering water by stomach tube in doses of 8 cc. per 175-grams rat at half-hour intervals. One such dose was given on the first day, 2 on the second, 3 on the third, 4 on the fourth, and 5 on the fifth. On the sixth day, 20 of these animals, along with 20 normal controls, were tested with 13 doses of water to confirm their resistance to water intoxication. The other 20 water-conditioned animals (table 2, group XV) were tested with 1.35 molar potassium chloride solution. Control animals for group XV were treated and tested by an identical procedure with the exception that a blank stomach tube was substituted for the administration of water (table 2, group XVI).

TABLE 2. *Toxicity of potassium to rats adapted to various non-specific factors and water intoxication*

GROUP	NO. OF RATS	ADAPTATION (ORAL)	TEST (ORAL)	SURVIVAL									
				1st dose		2nd dose		3rd dose		4th dose		Total	
				Min.[1]	%[3]	Min.	%	Min.	%	Min.	%	Min.[2]	Es[4]
XII	10♂	Blank tube	1.35M KCl	240	100	104	30	6	0			350	±33.9
XIII	10♂	H_2O	1.35M KCl	218	90	134	50	8	0			360	±50.9
XIV	9♂	1.35M NaCl	1.35M KCl	240	100	121	44.4	8	0			369	±41.0
XV	20♂	H_2O (Gaunt's method)	1.35M KCl	231	95	99	25	6	0			336	±27.6
XVI	10♂	Blank tube controls	1.35M KCl	240	100	119	40	5	0			364	±35.7

[1] Mean survival time in minutes following each test dose.
[2] Mean total survival time in minutes.
[3] Per cent of animals surviving four hours after each test dose.
[4] Standard error.

Group XII. The simple insertion of a blank stomach tube substituted for the potassium solution in the adaptation procedure.

Group XIII. An equal volume of distilled water substituted for the potassium solution in the adaptation procedure.

Group XIV. An equal volume of equimolar sodium chloride solution substituted for the potassium solution in the adaptation procedure.

Group XV. Adapted to water intoxication by the method of Liling and Gaunt.

Group XVI. A blank stomach tube was substituted for the administration of water in the adaptation procedure of Liling and Gaunt.

The increase in the resistance to potassium poisoning of both the water-tolerant animals and their blank tube controls, as compared with normal male controls (group I), was of slight significance, and of the same order as that afforded by the non-specific factors shown in table 2.

Effect of whole adrenal extract and desoxycorticosterone acetate on the resistance to the potassium ion. It is well known that adrenalectomized animals and patients with Addison's disease are highly susceptible to the potassium ion. Truszkowski and Duszynska (6) have shown that both whole adrenal extract and desoxycorticosterone acetate would protect normal mice against potassium poisoning. In light of these observations, it was of interest to determine whether adrenal extract or DCA would protect normal rats against potassium administered by our test procedure, and if so to what degree.

The whole adrenal extract employed in these investigations was prepared by the method of Cartland and Kuizenga as modified by Thatcher and Hartman (7), and was administered in a 10% alcohol solution, 1 cc. representing 300 grams of whole adrenal tissue. The synthetic DCA was also given in 10% alcohol, 1 cc. equaling 0.5 mgm.

The animals in these studies were divided into 4 groups. Two groups (table 3, groups XVII and XVIII) received subcutaneously either 1 cc. of the whole adrenal extract or of DCA 3 times a day at 6-hour intervals (8 A.M., 2 P.M., 8 P.M.) for 2 days before the test day. The other 2 groups (table 3, groups XIX and XX)

TABLE 3. *Toxicity of potassium to rats treated with whole adrenal extract, desoxycorticosterone acetate, and parathormone*

GROUP	NO. OF RATS	TREATMENT	TEST (ORAL)	SURVIVAL									
				1st dose		2nd dose		3rd dose		4th dose		Total	
				Min.[1]	%[3]	Min.	%	Min.	%	Min.	%	Min.[2]	Es[4]
XVII	20♂	Adrenal extract for 3 days	1.35M KCl	240	100	133	50	23	0			396	±30.7
XIX	20♂	Adrenal extract—test day only	1.35M KCl	240	100	125	40	19	5	1	0	385	±30.7
XVIII	18♂	DCA for 3 days	1.35M KCl	240	100	148	50	15	0			403	±28.1
XX	20♂	DCA test day only	1.35M KCl	240	100	83	25	5	0			328	±25.8
XXI	10♂	Parathormone	1.35M KCl	182	60	16	0					198	±30.1

[1] Mean survival time in minutes following each test dose.
[2] Mean total survival time in minutes.
[3] Per cent of animals surviving four hours after each test dose.
[4] Standard error.

Group XVII. Injected with 1 cc. whole adrenal extract three times a day for two days preceding the test day and one-half hour before each test dose of KCl on the test day.

Group XIX. Injected, on the test day only, with 1 cc. whole adrenal extract one-half hour before each test dose of KCl.

Group XVIII. Injected with 0.5 mgm. DCA three times a day for two days preceding the test day and one-half hour before each test dose of KCl on the test day.

Group XX. Injected on the test day only, with 0.5 mgm. DCA one-half hour before each test dose.

Group XXI. Injected with 0.5 cc. parathyroid extract (50 U.S.P. units) 18 hours previous to the first test dose of KCl.

were treated with either whole adrenal extract or DCA only on the test day. Treatment on the test day was the same for all 4 groups of animals, 1 cc. extract or DCA being administered one-half hour previous to each oral test dose of potassium chloride solution.

Both groups of animals treated with whole adrenal extract and the group pretreated with DCA (table 3) demonstrated a very significant degree of resistance to potassium poisoning as compared with normal control rats (table 1, group I). The *P* value of Fisher was found to be less than 0.01 in all 3 cases. However, when DCA was administered only on the test day (table 3, group XX), it offered little or no protection against the potassium ion.

It should be noted that there was no difference between those animals which

were pretreated with extract and those which received it only on the test day. On the other hand, the difference between animals pretreated with DCA and those treated only on the test day was a significant one (P slightly less than 0.05).

It can readily be seen that despite the massive doses of hormone or DCA the protection given by them is extremely small when compared with that shown by the potassium-adapted rats.

Effect of parathyroid hormone upon resistance to potassium poisoning. It is a well-known fact that the Ca^{++} ion can act as an antagonist to the K^{+} ion in certain physiological processes. The results of Winkler, Hoff and Smith (8) show that in dogs the toxicity of injected potassium was diminished by the simultaneous injection of calcium. In view of the action of parathyroid hormone in raising the concentration of the calcium ion in the serum, it was of interest to determine the effect of parathyroid extract upon the resistance of the rat to the potassium ion. Eighteen hours previous to the first oral test dose of potassium solution, one-half cc. of the parathyroid extract[2] (50 U.S.P. units) was administered. By inspection of table 3, it can readily be seen that the resistance of the rats treated with parathyroid hormone was very significantly less than that of control rats (table 1, group I) (P value of Fisher was found to be less than 0.01).

Attempt to produce an acquired tolerance to sodium chloride. The surprising observation of a true tolerance to the potassium ion suggested the possibility of such a phenomenon for the sodium ion. It was found that a test dose of 2.7 molar sodium chloride solution, administered according to the oral test procedure, would kill rats at a rate comparable with that for 1.35 molar potassium chloride. All attempts to increase the resistance of rats to this test, by substituting 2.7 molar sodium chloride for the potassium solution in the adaptation procedure, have thus far failed completely.

Both excess sodium chloride and water resulted in a prolonged period of sluggishness with depressed irritability terminating in death without convulsions.

In contrast, the premortal symptoms and manner of death of rats killed with the potassium ion were very characteristic. The symptoms, in order of appearance, were: *a*) loss of ability to stand on legs, *b*) jerky, uncoordinated movements, *c*) convulsions followed by a strong extensor thrust in the hind quarters, *d*) cessation of respiration, and finally *e*) cardiac failure. The heart continued to beat for approximately one minute after respiratory movements had ceased. The symptoms were the same regardless of the potassium salt used or the route of administration.

Discussion. In experiments which demonstrated that rats could be made tolerant to highly lethal doses of potassium salts, it was found that the tolerance was truly systemic and a specific one for the potassium ion. Animals made tolerant with potassium chloride survived test doses of potassium acetate; the converse was also found to be true. Moreover, animals adapted orally to potassium chloride and tested intraperitoneally demonstrated a highly significant tolerance compared with the intraperitoneal controls.

The increased resistance produced by the non-specific factors in the adaptation

[2] The parathyroid hormone was a commercial extract prepared by Eli Lilly and Company. Each cubic centimeter represented 100 U.S.P. units of parathyroid activity.

procedure was found to be extremely small compared with that produced by potassium adaptation. Likewise, an increase in resistance of the same low order was brought about by water adaptation. These two facts indicate that the tolerance to the potassium ion is a specific phenomenon and that it cannot be induced by increasing the resistance to water intoxication.

Contrary to expectations, the administration of parathyroid hormone resulted in a highly significant decrease in resistance to the potassium ion. It should be emphasized, however, that ions which are antagonistic to each other in relation to a given physiological process may act synergistically in another process (4). Odishima (9) has reported that the injection of potassium salt lowers the blood calcium, and vice versa. It is possible that the effect of parathyroid hormone upon the level of ionized calcium in the serum acts as an additional stress to the animals.

Either whole adrenal extract or DCA administered previously protects normal rats against potassium poisoning. This is in agreement with the results of similar work on mice by Truszkowski and Duszynska (6). The difference observed in the effectiveness of DCA and whole adrenal extract when administered only on the test day suggests a possible difference in the mode of action of the synthetic material and the natural hormone.

Although the protection against the potassium ion afforded by massive doses of whole adrenal extract or DCA was significant when compared to normal male controls, it was extremely small compared with that demonstrated by potassium-adapted animals, and of the same magnitude as that afforded by the non-specific factors or water adaptation. The fact that potassium-adapted rats showed a pronounced increase in water consumption accompanied by a profound diuresis in contrast to non-tolerant rats suggests that the primary potassium-tolerance mechanism is based upon a functional change in the kidney. It was of interest to note that animals treated with adrenal hormone or DCA, and those adapted to water intoxication or the non-specific factors, demonstrated no increased water consumption or diuresis following administration of potassium salts. This would seem to indicate that the protection afforded by adrenal hormone or non-specific stress was not upon an excretory basis and that the adrenal plays a direct rôle only in that portion of the tolerance mechanism which is non-specific in nature.

Nilson (10) points out that in order to bring about the crisis of adrenal insufficiency in adrenalectomized dogs maintained upon a low potassium, high sodium regimen, it was necessary to increase rapidly the intake of potassium, since such animals could adjust themselves to subtoxic doses of potassium within a few days and appear normal until the dose of potassium was again increased. This also suggests the presence of an extra-adrenal mechanism in the control of potassium metabolism, and indicates that a tolerance to potassium could be developed in the absence of the adrenal glands. Further studies in this direction are being pursued.

SUMMARY

1. A true systemic and a specific tolerance to the potassium ion was developed in rats by progressively increasing the size of doses of potassium salts adminis-

tered orally. A significant degree of this tolerance was found to persist for at least 7 days after cessation of the adaptation procedure.

2. The administration of whole adrenal extract or DCA brought about an increased resistance to the potassium ion, which was exceedingly small in comparison with that induced by potassium adaptation.

3. A difference was noted in the behavior of whole adrenal extract and DCA in that the former afforded immediate protection against potassium poisoning, whereas the latter did not.

4. The administration of parathyroid hormone resulted in a decreased resistance to potassium poisoning.

5. Attempts to develop a tolerance to sodium chloride failed.

6. It was observed that the manner of death produced by either excess sodium chloride or water intoxication was quite different from that caused by potassium poisoning.

The adrenal glands were furnished by Parke, Davis & Company, through the courtesy of Dr. Oliver Kamm; and the desoxycorticosterone acetate was supplied by Roche-Organon, Inc.

The authors wish to acknowledge their indebtedness to Dr. Frank A. Hartman for his encouragement throughout the course of this work.

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ADAPTATION OF THE ALBINO RAT TO DISCONTINUOUS CHRONIC EXPOSURE TO ALTITUDE ANOXIA

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Received for publication July 28, 1947

Armstrong and Heim (1, 2) in their pioneer work on the effects of altitude anoxia reported a decrement in the altitude tolerance of animals (rabbits) subjected to daily repeated exposure to anoxia. The initial response observed was beneficial adaptation for three to four weeks followed by deterioration of the adaptation, and of the general physical condition of the animals, until death terminated the experiment.

These findings were not confirmed by others, notably Thorn, Jones, Lewis, Mitchell and Koepf (3) who found that both rats and rabbits responded to mild repeated exposures to anoxia (18,000 feet equivalent) by apparently complete compensation. They did, however, find that the rabbit is not a good experimental animal for altitude experimentation because of problems associated with the gaseous content of the intestine in these animals. These workers explained the difference between their results and those of Armstrong and Heim on the basis of deleterious effects of the "altitude tolerance tests" used by the previous workers, which might well have caused sufficient damage to result in the deterioration noted.

Although this explanation appears to resolve the discrepancy in results, the possibility of injurious effects of discontinuous exposure to anoxia reflected in a decrease of anoxia tolerance is not eliminated, since Thorn, Jones, Lewis, Mitchell and Koepf did not test their animals for 'altitude tolerance'.

It seemed worthwhile to the authors to design an experiment aimed at clarification of this problem. Thus the present study was conceived in which a number of animals—sufficiently large to lend significance to the results obtained—were to be exposed discontinuously to anoxia mild enough to be easily tolerated (18,000 feet for one hour per day). At the end of a period long enough for regression to have started, if it were to occur, some of the animals would be given an altitude tolerance test and others would be examined for evidence of changes brought on by the chronic exposure.

METHODS. One hundred and two young (mean weight 207.5 grams) male, Sprague–Dawley albino rats were divided into four groups and paired according to body weight before the start of the experiment. Thus, *group 1* contained 26 marked animals slated for exposure to low pressure, and *group 3* contained 26 control animals, each one having the same body weight as an animal with

[1] Material for this paper was taken from a thesis submitted to the faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

the same ear marking in *group 1*. *Groups 2* and *4* were paired in the same manner, but each of these groups contained but 25 animals. Each group was then placed in a separate large wire cage and fed water and a dry, prepared pellet dog food ad libidum.

The first exposure of the test animals in the decompression chamber was made on the day following the pairing and repeated each day for 9 weeks. The exposure of *group 2* started one week prior to that of *group 1*, and the experiment was terminated for the former group one week earlier than for the latter. The advantage gained from this 'staggering' is represented by the fact that since the average weights of the two experimental groups (1 and 2) at any time represent the weights after the same length of exposure, but are a week apart chronologically, the effects of minor environmental changes on the average weights of the animals are partially eliminated.

Exposure of the experimental animals to simulated altitude was carried out in a large decompression chamber.[2] At approximately 5 P.M. every day, the entire rack of cages was taken to the chamber, the test animals put inside and the controls left outside. Ascent to 18,000 feet was made at a rate of 3000 feet per minute. The animals were left at this altitude for one hour with a ventilation of 30 cubic feet per minute, measured at ground level. Descent was made at 3000 feet per minute. After return to the animal room, the animals were given food and water in sufficient quantity to assure them an excess until the following exposure period.

All of the animals were weighed once a week on the afternoon of the same day of the week (Tuesday) as the original pairing. At the end of 9 weeks' exposure, the exposures were stopped and the animals chosen for study of organic effects were sacrificed. Thirty-five of the experimental animals taken at random and the corresponding controls were anesthetized with nembutal and dissected. The dissection procedure was as follows: The abdomen was opened and a 10 ml. hypodermic syringe containing a drop of heparin solution was introduced into the left ventricle of the heart by means of a #20 hypodermic needle. Blood was then drawn from the beating heart slowly until no more could be obtained. The volume of blood in the syringe was then recorded as an index to the total blood volume (two-thirds of total circulatory blood according to Selye, 4) and the blood used for erythrocyte count, using the Neubauer counting chamber, and for hemoglobin, using the Hellige–Wintrobe hemoglobinometer.

A small amount of air was then injected into the left ventricle and the heart was compressed between the fingers. This forced blood and bubbles of air into the aorta. Upon ceasing the compression, in the normal heart the air bubbles remain in the aorta in approximately the same position. However, in animals with insufficiency of the aortic valves, regurgitation occurs and the bubbles are seen to flow backward toward the ventricle. This was taken as an indication of valvular insufficiency.

The stomach was then removed, opened, washed free of contents and examined

[2] The decompression chamber used in this study is one on the grounds of the National Institute of Health, Bethesda, Maryland, and is the property of the U. S. Public Health Service (Industrial Hygiene Research Laboratory).

for blood and for gastric ulcers. These are plainly visible, when present, as hemorrhagic spots, or as small lesions surrounded by an elevated area. The carcass of the animal was then examined for anatomical abnormalities, and records kept of those found. After the dissection of 70 animals, 32 rats remained alive. These animals were used for altitude tolerance tests. Twelve of these animals (6 experimental, 6 control) were subjected to acute anoxia by decompression to an equivalent of 50,000 feet (87 mm.Hg) on the day following the last acclimatizing exposure. 'Ascent' was performed very slowly (2 hours) to reduce the possibility of decompression sickness. Twenty animals (10 experimental, 10 control) were allowed to rest at ground level in the animal room for

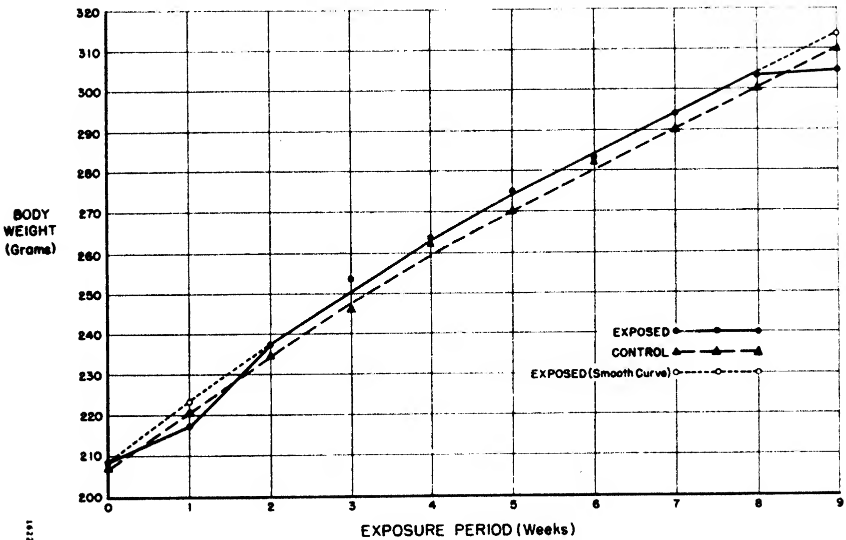

FIG. 1. Growth of rats exposed to altitude

one week after the last exposure to 18,000 feet, and then exposed to acute anoxia. Records of the survival time and apparent cause of death for each animal were made during these acute exposures.

Statistical analysis of numerical measurements was performed utilizing the Fisher t-test (5) to show reliability of differences between test and control-group averages.

OBSERVATIONS. 1. *General condition*. Little difference was noted between the general physical condition of the animals exposed to 18,000 feet simulated altitude and that of the animals maintained at sea level during the course of the experiment. The average body weight of the exposed animals closely paralleled that of the control animals throughout the 9 weeks' test period. A graphic presentation of the average body weights of the two groups is presented in figure 1. No statistically reliable difference is observed between the control and exposed group points for any weekly weight mean. However, it will be noted

that at two points (1 week and 9 weeks) the mean weight of the exposed animals falls below the mean weight of the controls, whereas at every other point the mean weight of the exposed animals is uniformly above that of the controls. While the numerical difference between the mean weights of the exposed and control groups do not differ significantly at these points, the deviation of the exposed group mean weight from the mean weight expected for that group on the basis of a smooth growth curve is significant at the 5% level. No other point on either curve is significantly deviant from the corresponding theoretical point on a smooth curve.

TABLE 1. *Effects of discontinuous exposure to 18,000 feet for 1 hr/day for 9 weeks on the albino rat*

	CONTROL ANIMALS		EXPOSED ANIMALS		CHANGE†	P‡
	Datum*	No.	Datum*	No.		
					%	
Blood volume (ml.)	6.86	37	8.28	34	20.7I§	.001
Red cell count (million/mm^3)	8.02	37	8.04	35	0	>.50
Hemoglobin (grams %)	15.2	35	17.4	35	14.5I	.02
Total O_2 capacity	2.1	35	2.9	35	38I	.02
Spleen wt. (grams)	0.5900	27	0.5947	27	0	>.50
Aortic valve insufficiency	1 (2.9%)	35	12 (34.3%)	35	91.7I	.0001
Gastric ulcers	7 (20%)	35	15 (42.9%)	35	53.3I	.03
Pulmonary lesions	3 (8.6%)	35	16 (45.7%)	35	81.3I	.0001
Survival under acute anoxia¶	2/6	6	2/6	6	0	>.50
Survival under acute anoxia‖	8/10	10	3/10	10	62.5D	.03

* Mean of all observations.

† Percentage difference between exposed and control groups in terms of normal (control value).

‡ Probability of observed difference occurring by chance as determined by Fisher t-test.

§ I = increase; D = decrease.

¶ Exposure to acute anoxia (50,000 ft. equivalent) the day after completion of 18,000 feet exposures.

‖ Exposure to acute anoxia one week after completion of 18,000 feet exposures.

It is interesting to note that these deviations occur at the points where a decrement in weight gain would be expected under conditions of more severe anoxia, the one-week decrement representing a lag in adaptation and the 9-week decrement usually interpreted as a recession of adaptation.

After two weeks of exposure, the ears and the plantar surfaces of the feet of the test animals were found, practically universally, to be slightly darker in color than those of the controls, indicating that adaptation in circulating hemoglobin had occurred by this time.

2. *Effects on specific systems.* A summary of the effects of exposure is given in table 1.

A. *The blood and circulatory system.* Blood volume was found to be appreciably increased in the animals exposed to 18,000 feet altitude. As is shown in the accompanying table, an increase of approximately 21% occurred.

The erythrocyte count of the exposed animals was not significantly different from that of the controls. The average counts of both groups, 8,020,000 and 8,040,000, respectively, are within the normal range for albino rats of the age group represented (approximately 20 weeks). The hemoglobin content of the blood, however, was significantly increased (14.5%).

Insufficiency of the aortic semilunar valves was found in 34.3% of the exposed animals examined, while only one of the corresponding 35 control animals (2.9%) showed this deficiency. It is possible that this one represents experimental error, in that rupture of these valves might possibly be effected during the dissection technique.

The mean weight of the spleen was not changed significantly by the exposure to altitude.

B. *General pathology.* (1) Gastric Ulceration. The incidence of gastric ulcers was appreciably higher in the animals exposed to altitude (43%) than in the control animals (20%). All cases of actively hemorrhagic gastric ulcers encountered were in the experimental group.

(2) Pulmonary Lesions. The occurrence of lesions of the lungs was much more common in the exposed animals (16 cases in 36) than in the control animals (3 cases in 35).

3. *Resistance to acute anoxia.* A. Of 'acclimatized' and control animals subjected to acute anoxia the day after the last regular exposure, the resistance to high altitude (50,000 feet) was identical in each group (exposed simultaneously). Four out of 6 animals from each group died with convulsions over the same exposure period.

B. When kept at sea-level pressures for one week after the last regular exposure to 18,000 feet, it was found that mortality in the previously treated animals was greatly increased upon exposure to acute anoxia. Thus, of 10 test animals subjected to very high altitude, 7 died, while of 10 control animals exposed at the same time, only 2 did not survive.

Discussion. The increase in blood volume of animals exposed to altitude reported here has been observed previously by Dalton (6). The value of this increase to the organism under conditions of low oxygen pressure is obvious. Also, the increase in hemoglobin observed permits of a greater oxygen-carrying capacity per unit volume of blood. This increase has also been reported by Van Liere and Stickney (7) for dogs, and by Campbell (8, 9). However, for humans native to high altitude (c. 15,000 feet) Hurtado (10) reports no elevation of average hemoglobin content of the blood.

A direct analysis of known factors in the present study indicates that the total oxygen-carrying capacity of the blood was increased in the exposed animals to 2.9 ml. as compared with a total capacity of 2.1 ml. for the controls, an increase of 38%. However, so many unknown factors (cardiac output, vasomotor tone, respiratory regulation of blood and tissue pH, etc.) are involved in the effect of

this increased O_2-carrying power on the total organism that this does not present by any means a reliable index of tissue oxygenation.

It appears inconsistent that the erythrocyte count was not correspondingly increased. In fact, Van Liere and Stickney (7) report an increase of 84% in erythrocyte count of dogs exposed to 18,000 feet for 17 weeks and Thorn *et al.* (3) report a similar increase in both hemoglobin per cent and red cell count for dogs exposed in the altitude chamber. However, the benefit to the organism of increase in hemoglobin content of the blood without recourse to increase in the number of erythrocytes is obvious, since an increase in the number of red cells is accompanied by a corresponding increase in the viscosity of the blood, as shown by Hurtado (10). This is decidedly a detrimental factor because of the increased work demanded of the circulatory system.

A number of workers have studied the effect on the heart of low barometric pressure. Van Liere (7, 11) was unable to produce cardiac hypertrophy (measured by change in weight) in guinea pigs at 20,000 feet altitude either when exercised or at rest, nor in dogs at 18,000 feet. This, however, does not show that dilation or valvular rupture do not occur. In fact, Campbell (8) and Barach (12) have both reported symptoms of congestive failure in animals exposed to 20,000 feet altitude, in the former case associated with cardiac enlargement. Also Dalton (6) has observed histologically, demonstrable damage to the A-V valves in rats exposed discontinuously to 25,000 feet, and Nims (13) has recently reported cardiac lesions in rats produced by a single short exposure to a simulated altitude of 20,000 feet.

The fact that the heart is placed under a considerable strain under the conditions of the present experiment is borne out by the fact that damage of the aortic valve was found to have occurred in approximately one-third of the experimental animals. In this group the average hemoglobin percentage (20.6 grams %) is well above the average for the entire experimental group (17.4 grams %), but the average red cell count (8,400,000) was very near the average of the experimental group (8,030,000). It is possible that the elevated hemoglobin found in the animals with valvular insufficiency represents an attempt on the part of the organism to nullify the cardiac defect. It seems very likely that the symptoms of congestive failure reported by Campbell (8) and Barach (12) are due to valvular damage produced by the anoxic experience.

It is evident that adaptation to mild anoxia enables the organism to resist exposure to acute anoxia at least as well as can the normal animal, but when any advantages this adaptation may have imparted have 'passed off', as shown by Campbell (8) to occur during a stay at low altitude, the organism is less fitted to resist extremely high altitude than is the normal animal. This phenomenon may conceivably be due to deleterious changes in the circulatory system incurred during the original adjustment to low pressure.

The lack of increase in spleen weight in the acclimatized animals is probably correlated with the lack of increase of circulating erythrocytes, as reported above. Studies made at 18,000 feet, 8 hours per day for 3 months by Van Liere and Stickney (7) have shown an increase in spleen weight of dogs along with an increase of red cell count.

No reference to disturbances of the respiratory tract as a result of chronic exposure to anoxia has been found in the literature. The results found in the present study indicate that some phase of the effects of exposure to low barometric pressure—possibly hyperventilation, pulmonary occlusion, increased arterial pressure or a combination of these factors and others—have a deleterious effect on the lung tissues.

It appears evident from the above findings and review that at least two counteracting forces are present during the course of adaptation to altitude anoxia. One is the direct damage caused by the anoxic experience, as shown by Nims (13) to occur during the earliest exposures. The other is the beneficial process of adaptation, which more or less typifies the 'alarm reaction' of Selye (4).

To interpret the findings discussed above with reference to the phases of the 'alarm reaction,' it is necessary to reconsider them together.

The phase of acute alarm is engendered in the organism exposed to low barometric pressure, probably during the first few exposures. The beginnings of the adaptative response to anoxia (i.e., adrenal hypertrophy, hemoconcentration, etc.) can be demonstrated after a single exposure (Thorn, 3).

The adaptation phase is commonly referred to as 'acclimatization' when it results from exposure to low pressure. However, if acclimatization is taken to mean that its result is an organism more fit to survive under anoxic conditions than is the normal animal, it is seriously doubted by the author that this occurs to any practical degree in discontinuous exposure to anoxia. Certainly it does not occur in the present study as is evidenced by the exposures to acute anoxia cited above. Any beneficial adaptative response which had occurred in the exposed rats was only sufficient to counteract the deleterious changes in the circulatory system. In fact, if the animals were allowed to lose the beneficial adaptative changes by a week's stay at ground level, the deleterious effects remaining were sufficient to cause an appreciable decrease in the altitude 'ceiling' of the test animals as compared with the normal controls. Thus, it seems probable that no practical benefit to the organism can be expected from acclimatization by discontinuous exposure to even a mild degree of anoxia.

The appearance of regression of adaptation, of course, still further embarrasses the organism exposed to anoxia, since a part of this adaptation has been utilized in counteracting the previously imposed pathology and only part has been effective in 'prophylaxis' against further injury. Regression of adaptation is strongly suggested in the present findings, although the slight decrement in weight-gain found after 9 weeks of exposure does not alone confer great confidence in this conclusion.

In general, it is the belief of the authors that the original findings of Armstrong and Heim (1, 2) are confirmed by the present study. Discontinuous exposure to altitude anoxia leaves the exposed animal, after loss of adaptation, in a poorer condition to cope with anoxia than the control animal. However, in accordance with Thorn *et al.* (3), the use of altitude tolerance tests by Armstrong and Heim during the period of adaptation, no doubt contributed toward the weakening of the animal and in a measure intensified the final results.

SUMMARY

1. Exposure of 51 albino rats to discontinuous anoxia by decreased barometric pressure, equivalent to 18,000 feet for one hour per day for 9 weeks, produced no obvious changes in appearance, weight or behavior as compared with 51 carefully paired control animals.

2. Statistical analysis of changes occurring showed the concomitant occurrence of beneficial adaptative changes and deteriorative changes during the exposure period.

3. The exposed animals were found to have a decreased resistance to acute anoxia (50,000 feet equivalent) after loss of adaptation, as compared with the normal control animals.

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REDISTRIBUTION OF POTASSIUM, SODIUM AND WATER IN BURNS AND TRAUMA, AND ITS RELATION TO THE PHENOMENA OF SHOCK[1]

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Received for publication July 26, 1947

The outstanding clinical feature of shock from severe burns or trauma is circulatory collapse (1) which is presumed to result from fluid loss at the site of injury (2). Recent experimental (3, 4) and clinical (5, 6) studies of the treatment of shock from burns and trauma have indicated that effective replacement therapy requires a volume of sodium-containing fluid greatly in excess of any loss at the site of injury. Since whether or not this extra fluid was administered apparently produced the difference between success and failure, it seemed important to ascertain the nature and extent of the fluid and electrolyte shifts that occur in injured and in uninjured tissues. Such tissues, therefore, were analyzed for potassium, sodium and water. In addition, total protein and electrophoretic analyses of these tissues were made (7) to characterize the quantity and nature of the 'protein loss'. The data obtained provide evidence that in traumatic shock, primary changes in the tissues lead to reduction in both blood and extracellular volumes followed by circulatory collapse and death.

Methods. Traumatic shock was produced with Rosenthal's technique (4). The tourniquet which completely stopped the circulation was applied for two hours, then removed; the leg swelled and promptly the animal exhibited marked shock. It should be noted that while the application of tourniquets to both hind legs resulted in an injury that was fatal if untreated (4), a single leg tourniquet did not. Nevertheless the use of a single tourniquet produced symptoms of marked shock and possessed the advantage of supplying an injured and an uninjured 'control' hind leg on the same animal. It will be shown, however, that the contralateral uninjured leg undergoes considerable changes and is not identical with the leg of a normal animal. Animals were sacrificed by bleeding from the heart from 4 to 6 hours after removal of the tourniquet. Data were obtained in the same way for normal mice and mice deprived of food and water for like periods.

For analysis the legs were amputated at the groin by cutting across the neck of the femur with a scissors and the entire leg was taken as the sample[2], thereby

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Columbia University College of Physicians and Surgeons. The paper was read in part before the Society for Experimental Biology and Medicine, December 16, 1944.

[2] The degree of anatomic reproducibility of this sampling is indicated by comparison of the dry weight of right and left leg of each animal (table 1A). The tissue weights were not averaged because of considerable variation in the size of the mice used.

TABLE 1. *Sodium, potassium and water content of entire legs of uninjured mice and those whose legs had been traumatized by the application of a tourniquet*

ANIMAL NO.	WEIGHT OF EACH LEG		WATER CONTENT	SODIUM	POTASSIUM	Na+K
	Wet tissue	Dry				
Uninjured mice; deprived of food but not water						
	grams	*grams*	%	*mEq. per kgm. of H_2O*		
1	1.149	0.363	68.4	94.5	118.6	213.1
	1.224	0.337	69.2	87.8	119.1	206.9
2	1.036	0.337	67.5	88.9	122.5	211.4
	1.162	0.377	67.6	89.2	119.0	208.2
3	1.024	0.320	68.8	90.0	118.5	208.5
	1.120	0.347	69.0	88.6	118.0	206.4
4	0.989	0.307	68.9	93.5	120.0	213.5
	1.091	0.338	69.1	92.1	124.0	216.1
Shocked mice: traumatized legs 4 hours after injury						
5	1.179	0.283	76.0	160	47	207
6	1.659	0.361	78.2	158	38	196
7	2.137	0.473	77.8	152	39	191
8	1.959	0.463	76.3	150	40	190
9	2.188	0.518	76.4	151	41	192
10	2.52	0.565	77.6	151	39	190
Shocked mice: opposite uninjured legs						
5	0.914	0.312	65.8	80.6	135	215.6
6	1.023	0.333	67.5	74.4	124	198.0
7	0.910	0.291	68.0	—	—	—
8	1.006	0.309	69.1	81.4	125	205.4
9	1.062	0.361	66.1	81.2	133	214.2
10	1.378	0.437	68.2	69.6	129	199.0
Uninjured mice: deprived of food and water for same period						
11	1.109	0.339	69.4	90.5	127	217.5
	0.936	0.291	69.0	96.5	124	220.5
12	0.958	0.317	66.7	93.6	126	219.6
	1.147	0.409	64.3	94.4	124	218.4
13	1.076	0.349	67.6	84.5	128	212.5
	1.099	0.346	68.5	79.6	126	205.6
14	1.179	0.380	67.7	81.6	128	209.6
	1.154	0.383	66.8	85.6	131	216.6

eliminating large errors due to unavoidable loss of fluid when sampling muscle or skin of the very edematous injured area. Water was determined by drying for a week or more to constant weight in an oven at 105–110°C. The dry tissues were then extracted with 0.75 N nitric acid (8).

Radioactive isotopes were given intraperitoneally or subcutaneously the day before the experiment; with radiopotassium, an additional injection was given two days in advance. Radioactivity was measured in aliquots of those solutions with a Geiger-Muller counter.

Potassium and sodium were determined in the improved internal standard flame photometer (9) by adding a constant amount of lithium nitrate to all samples and obtaining the ratio of potassium or sodium to lithium. Repeated readings indicated a reproducibility of 0.5%. By addition of lithium, an overall precision of 1% is obtained despite considerable variation in the concentrations of other ions or organic substances (9). It is of the utmost importance to distinguish this instrument from those not using internal standards, since such instruments are affected by variations in the composition or ionic concentration of the solutions which markedly elevate or depress the readings (9). The maximal accuracy of the direct reading photometer (10) is 3% after 10 readings with standard and unknown solutions of similar composition; the accuracy of a commercial model[3] in our hands was only about 10% and this instrument therefore was not employed. In one experiment, tissue sodium was determined by a uranyl zinc acetate method after dry ashing (11).

Results in traumatic shock. The data in table 1 are expressed in terms of the concentration of potassium and sodium in the water of the entire leg thus showing whether total tissue fluid was diluted or concentrated and whether the relation of potassium and sodium was altered. While, in the main, potassium is the chief intracellular cation and sodium the chief extracellular cation (12), recent studies have indicated frequent interchange in disease (13). Data expressed in terms of total tissue water avoid any assumption as to location within or outside of cells; nevertheless, absolute as well as relative changes are indicated.

The traumatized tissues gained considerable water; their potassium concentration was reduced by two-thirds and their sodium concentration was almost doubled. The opposite uninjured legs surprisingly did not lose water when compared to control legs but showed an increase in potassium and an equivalent decrease in sodium concentration. These changes are more conspicuous when the values are compared with those of normal animals deprived of food but not water. The analytical results of several experiments are summarized in table 1B and agree with the typical experiment given above.[4]

[3] Made by Perkin-Elmer Co., Glenbrook, Conn. (Model 18)

[4] In 3 additional experiments, before the internal standard technique had been adopted, it was possible to measure only the *ratio* of Na to K because the strong acid used to ash the tissues for nitrogen analyses depressed the absolute readings, but since Na and K are similarly affected their ratio values were reliable. The ratios obtained in normal, injured and the opposite legs were practically the same as those given in table 1B. We are indebted to Dr. John Berry and Mr. David Chappell of the Stamford Research Laboratories of the American Cyanamid Co. for these and many other of the earlier measurements.

TABLE 1B. *Summary of average values of three experiments*

EXPERIMENT NO.		NO. OF LEGS	WATER CONTENT	SODIUM	POTASSIUM	Na+K	RATIO, mEq $\frac{Na}{K}$
			%	*mEq. per kgm of water*			
6[1]	Uninjured with H_2O but no food	8	68.6±0.6[3]	90.7±2.5	120±2	210.5±4	0.75
7	Uninjured mice legs	8	67.5±1.5[2]	88.0±6	127±2	215±5	0.694
	Shocked mice						
	Injured legs	6	77.0±0.8[3]	154.0±3	41±3	194±6	3.75
	Opposite legs	5	67.5±1.2	77.0±5	129±4.5	206±7	0.597
4	Uninjured mice legs	6	58.9±3.7[2]	81.0±3	126±3	208±3	0.644
	Shocked mice						
	Injured legs	6	75.1±1.8	142.0±2	48±5	191±4	2.96
	Opposite legs	6	63.3±1.4	77.0±4	133±3	211±4	0.578
2	Uninjured mice legs	4	60.1±0.2[2]	90.0±7.4	127±2	217±9	0.71
	Shocked mice						
	Injured legs	4	74.1±1.7	153.0±1.6	45±3	199.5±2	3.4
	Opposite legs	3	62.0±0.9	78.0±3.9	132±2	210±2	0.59

[1] Individual values given in table 1.

[2] There is considerable variation in water content of tissues of uninjured control mice because of varying degrees of water loss with dehydration which did not occur in injured mice (34).

[3] Standard Deviation.

TABLE 2A. *Redistribution of sodium in tissues in tourniquet shock traced by radiosodium (Na^{24})*

EXPT. NO.	NORMAL MICE		SHOCKED MICE			
	Both hind legs		Injured legs		Opposite uninjured legs	
	No. legs	averaged	No. legs	averaged	No. legs	averaged
I	4	100	4	230	4	79
II	12	100	10	236	10	83
III	4	100	6	405	6	84

(Values are % radioactivity per gram dry weight of tissue.)

Similar experiments employing radioactive isotopes are summarized in tables 2A and 2B and show that the potassium and sodium shifts (14) traced by these isotopes are the same as those indicated in table 1. Radiobromide was used as

TABLE 2B. *Redistribution of potassium in rat tissues[1] in tourniquet shock traced by radiopotassium-comparison with direct analyses*

5 shocked rats

RAT NO.	RADIOPOTASSIUM PER GRAM HIND LEG		RATIO $\frac{\text{INJURED}}{\text{OPPOSITE}}$	DIRECT ANALYSES (MEQ. PER GRAM LEG) RATIO $\frac{\text{INJURED}}{\text{OPPOSITE}}$
	Injured	Opposite		
1	2.06	4.2	.49	.50
2	2.48	4.4	.56	.54
3	1.73	4.32	.40	.31
4	2.08	4.21	.49	.47
5	2.11	3.74	.56	.55
Average	2.09	4.18		

3 normal rats

	RIGHT	LEFT	R/L	R/L
6	3.08	3.16	.97	.98
7	4.4	4.6	.96	.95
8	3.74	3.73	1.0	1.01
Average (of six legs)				3.79

[1] Radiopotassium was given in 2 subcutaneous injections 24 and 48 hours preceding application of tourniquets. Because of shortage of mice when the isotope was available, 30-gram rats were used.

[2] These values are the % per gram of hind leg of the total radioactivity present in each rat (both legs and the remaining carcass) at autopsy.

TABLE 3. *Redistribution of sodium and water in muscle and 'skin' in tourniquet shock; effect of sodium therapy—gravimetric analyses*

	MUSCLE		'SKIN'	
	Sodium	Water	Sodium	Water
	mEq/kgm. H_2O	%	*mEq/Kgm. H_2O*	%
Normal mice (4 hind legs)	52	74.4	204	54.0
Shocked mice[2]				
(4 injured legs)	149	86.7	223	72.2
4 opposite uninjured legs	39	73.8	136	57.0
Shocked mice—saline therapy[3]				
4 injured legs	180	85.3	162	85.4
4 opposite uninjured legs	48	73.8	164	61.5

[1] 'Skin' refers to cutis and subcutis including hair.

[2] Tissue taken for analysis 4–6 hours after release of tourniquet.

[3] Treatment consisted of 2 cc. of 0.9% NaCl given intraperitoneally one hour after release of tourniquet; tissue taken for analysis 3–5 hours later.

TABLE 4. *Sodium, potassium and water content of entire hind legs burned and not burned*

ANIMAL NO.			WEIGHT OF EACH LEG		WATER CONTENT	SODIUM	POTASSIUM	Na+K
			Wet tissue	Dry				
A. Right hind leg immersed in water at 75 C. for 10 seconds								
			grams	*grams*	%	*mEq. per kgm. H_2O*		
1			1.259	0.292	76.8	144	58	202
2			1.497	0.346	77.0	141	52	193
3			1.231	0.280	77.3	141	60	201
4			1.659	0.362	78.2	145	48	193
5			1.472	0.334	77.4	143	53	196
Opposite hind leg—not burned								
1			0.972	0.332	65.8	77	143	220
2			0.982	0.326	66.8	80	143	223
3			0.913	0.292	68.0	75.4	143	218
4			1.022	0.340	66.8	78.1	127	205
5			0.933	0.310	66.8	82.5	140	222
B. Right hind leg immersed in water 94–99 C.								
	°*C*	*sec.*						
1	94	60	1.204	0.397	67.0	100.5	85.4	186
2	94	60	1.609	0.476	70.4	118.1	66.2	184
3	96	60	1.325	0.410	69.1	105	88.9	194
4	98	45	1.245	0.383	69.2	82.4	108.6	191
5	99	45	1.260	0.373	70.4	82.4	94.4	177
6	94	30	1.297	0.413	68.1	126	92.5	219
7	96	30	1.147	0.410	64.2	134.6	88.3	223
8	96	30	1.264	0.382	69.8	119	90.7	210
9	98	30	1.230	0.397	67.7	134	84	218
Opposite hind leg—not burned								
1			1.643	0.545	66.8	94.6	115.8	210
2			1.293	0.456	64.7	89.6	135	225
3			1.079	0.375	65.2	84.4	134.5	219
4			1.052	0.339	67.8	77.2	128	205
5			1.014	0.299	70.6	81.9	117.4	199
6			1.243	0.383	69.2	74.7	108	183
7			1.034	0.374	63.9	73.9	144	218
8			0.928	0.315	66.0	82.6	146.8	229
9			0.986	0.325	67.0	70	141.9	212

C. Average of values in B on dry weight basis (*mEq. per kgm. dry weight*)

	Na	K	Na+K
Uninjured mice legs	163	250	412
Burned legs (difference).....................	241 (+78)	171 (−79)	412
Opposite legs..................... (difference).....................	158 (−5)	262 (+12)	420 (+8)

tracer of chloride[5] in two experiments which showed that the movement of sodium into injured tissues is accompanied by a similar shift of halide.

In one experiment muscle and skin were analyzed separately for sodium (11) (table 3). The normally low content of sodium in muscle increased greatly after injury; in contrast, the normally high sodium in skin increased but slightly after injury. In the opposite uninjured leg, the sodium concentrations of both tissues were reduced; these low values were raised after therapy with 0.9% sodium chloride solution.

RESULTS IN BURN SHOCK. In tissues burned by immersion in water at 75°C. for 10 seconds the redistribution of potassium, sodium and water (table 4A) closely resembled that after tourniquet trauma. In sharp contrast, after immersion in water at 94–99°C. for 30 to 60 seconds (table 4B) no extensive accumulation of water occurred in the burned leg (16). Nevertheless the burned leg lost potassium and gained sodium and the opposite uninjured leg conversely gained potassium and lost sodium. Although scant edema resulted, the constance of the value for Na + K shows that the potassium lost by the burned leg equalled the sodium gained. This is most apparent when the averaged values on a dry weight basis are compared (table 4C)

DISCUSSION. The several analytical methods employed gave essentially the same results, summarized graphically in figures 1 and 2. The results are expressed in terms of the dry weights for direct comparison. The protein analyses[6] are included to give a more complete picture. Our analyses of injured tissues show complete agreement with those reported by Tabor and Rosenthal using chemical methods (17, 18). The tissue analyses of Ricci *et al.* (19) are given in terms of deviation from the opposite leg and the general direction of their results is similar. Insofar as it is possible to compare the analyses of injured tissues and edema fluid made by Brues, Zamecnik, Aub *et al.* (20), an influx of sodium and water into injured tissues and an outward movement of potassium are apparent. Bollman *et al.* (21) measured phosphate in injured tissues and described a decrease compared to the uninjured tissues analagous to our potassium data. It is of interest that Blalock (2) found that the concentration of chloride in the supernatant centrifuged from injured tissues was similar to the concentration in the plasma.

It is important to recognize by reference to analyses of normal skin (22) and to table 3, that if the injury is confined to the skin which contains little potassium, or after more extensive injury, if the samples taken for analysis do not include muscle, extensive potassium changes will not be found and less striking changes in sodium concentration will be observed.

[5] Radiochloride decays very rapidly and since bromide has been shown to be similarly distributed throughout tissues (15), minute amounts of relatively longer-lived radiobromide serve well as a chloride tracer.

[6] The total protein content (nitrogen by Kjeldahl x 6.25) of edematous injured legs was found equal to that of opposite uninjured legs. Edema resulting from an influx of plasma might be expected to increase the protein content by 10%. Electrophoretic analyses of leg edema-fluid disclosed components other than those characteristic of plasma proteins (7), i.e. no plasma albumin appeared in the injured leg.

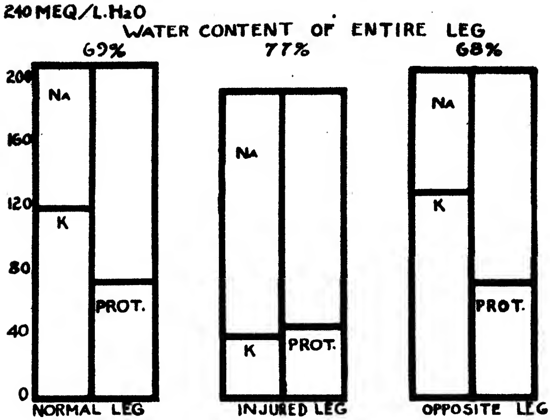

FIG. 1. *The redistribution of potassium, sodium and water after trauma to one leg of mice.* Values for Na and K are expressed in milliequivalents per kilogram of water. The values for protein are 31.1±1.7, 18.5±1.6 and 30.7±1.7 grams per 100 grams of water in normal, injured and uninjured opposite legs, respectively.

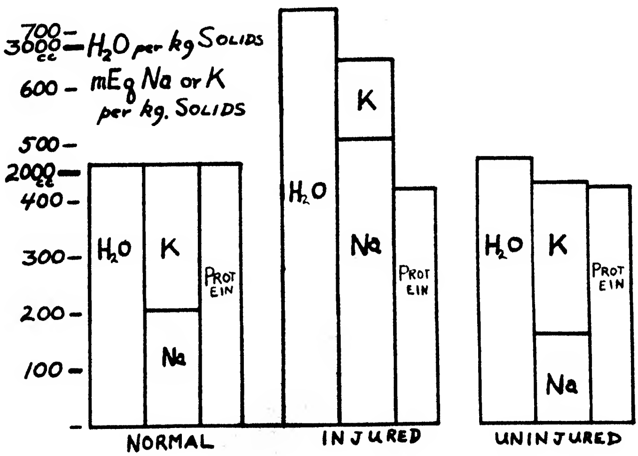

FIG. 2. *Potassium, sodium and water content of tissues after trauma to one leg of mice.* Values for Na and K are expressed in milliequivalents per kilogram of dry weight, water in cc. per kilogram of dry weight. The values for protein are 690, 620 and 650 grams per kilogram solids in normal, injured and uninjured opposite legs, respectively.

In view of this agreement in data from several laboratories[7] and, to our knowledge, the absence of conflicting data, it is important to consider the sig-

[7] Dr. Holmes has very kindly supplied us with the wet and dry weights of the dog legs analyzed in a recent study of traumatic shock (35). When these Na and K analyses are calculated per kgm. dry weight or per kgm. water, the values are in good agreement with our values.

nificance of these findings.[8] The gross swelling of the injured leg attests the movement of fluid into the region. As seen in table 5, an influx of plasma or interstitial fluid sufficient to account for the gain of 0.51 grams of water is insufficient to account for the electrolyte changes. After allowing for such an influx, however, there is an excess of sodium which approximates the decrease in potassium, suggesting that an exchange of Na from extracellular fluid for intracellular K occurred. Thus, injury caused not only edema but also affected the metabolic activity of cells so that they no longer maintained the integrity of their cationic structure. Synoptically three events occur: *a*) accumulation of extracellular fluid as edema in the region of injury; *b*) loss of considerable potassium from injured tissue cells; *c*) exchange of sodium from extracellular fluid for intracellular potassium extruded.

The systemic effects of the first two events are reflected in the uninjured legs by their decrease in sodium and their increase in potassium. The effects of the third event are indirect. Removal of sodium without water from extracellular

TABLE 5. *Electrolyte and water content of normal and injured leg*

	WATER	SODIUM	POTASSIUM
	grams	*mEq.*	*mEq.*
Normal leg	0.79	0.075	0.094
Injured leg	1.30	0.205	0.049
Difference	+0.51	+0.130	−0.045
Influx of ECF equal to gain in water	0.51	0.077	0.002
Unaccounted for	0	+0.053	−0.047

fluid reduces its sodium concentration[9] (and total cation) and hence lowers its osmotic pressure. Uninjured cells imbibe water from this hypotonic fluid (25) just as erythrocytes swell when placed in hypotonic saline solution. This swelling of cells of uninjured tissues, combined with the decreased sodium content and hence reduced volume of extracellular fluid in such regions, accounts for the apparent contradiction that the water content of uninjured tissues is not significantly reduced despite the large amount of fluid gained by the injured region.[10] Finally, hydration of cells very likely interferes with their function

[8] After injury by exposure to freezing temperatures, similar shifts of Na and H_2O have recently been reported (23).

[9] This shift of Na without equivalent amounts of water accounts for the observation that the Na concentration of venous plasma draining a burned limb is lower that the Na concentration of the entering arterial plasma (24). Plasma Na was decreased after severe burns (5, 34) and after bilateral tourniquet injury (34) when loss of Na into injured tissues exceeded removal of water by cells.

[10] A possible source of water is suggested by the negligible insensible weight loss of injured mice during a 6-hour interval in which uninjured mice without food and water lose about 10 % of body weight by insensible routes (34). Metabolism in shocked mice is markedly reduced (36).

and the water acquired by cells represents a definite loss of extracellular fluid volume.

Release of potassium from injured tissues is well known but the question of its toxicity has remained *sub judice* because the terminal plasma potassium concentration in shock is below that observed in normal animals poisoned with potassium (17). However, Tabor and Rosenthal in a thorough investigation (17, 18) found that shocked animals exhibit a tenfold increase in sensitivity to injected potassium but not to other toxic substances. In addition, they found that in shocked rabbits killed immediately by injection of potassium, the plasma K was lower than in normal rabbits killed by K injection but similar to the terminal plasma-K-concentration in shock. Apparently after trauma, the resulting increase in potassium in uninjured tissues greatly augments the danger of its increased concentration in the plasma. It is noteworthy that injection of potassium salts into normal animals with isotonic extracellular fluid does not lead to increase in the potassium concentration of tissue water (25) but the simultaneous availability of excess potassium and hypotonic extracellular fluid in shock seems to permit water and excess potassium to enter uninjured tissue cells. Similarly, in adrenalectomized rats with decreased concentration of plasma sodium, there is an increased concentration of potassium in tissue cell-water and a specific sensitivity to potassium (26).

Inasmuch as the outstanding clinical manifestation of shock is circulatory collapse (1), the possible influence on the circulation of these changes in the tissues warrants consideration. The accumulation of extracellular fluid in the injured leg can be expected to decrease the volume of circulating fluid. Although this is the usual explanation for the decrease in plasma volume (2), Scott (27) found that the volume of fluid that accumulated in the injured region could not be correlated with death or survival. The significantly greater volume of fluid required for successful replacement therapy (3, 4) also points to a loss of extracellular fluid in addition to that occurring in the region of injury. Such additional reduction in extracellular fluid volume is revealed by these tissue analyses as occurring in the uninjured tissues by virtue of their imbibition of water from the hypotonic extracellular fluid (resulting from the third event described above). Thus, in table 5 the 0.053 mEq. of sodium lost in damaged cells (above that in the 0.51 gram of fluid gained by the injured leg represents approximately 0.3 gram of extracellular water $\left(\frac{0.053 \text{ mEq.}}{0.15 \text{ mEq. Na per gram}}\right)$ which is imbibed by the uninjured tissues, together with potassium extruded from the injured cells. In this manner the extracellular fluid volume is reduced by two events occurring in the tissues: *a*) accumulation of extracellular fluid as edema at the site of injury, as emphasized by Blalock (2); *b*) imbibition of water and potassium by uninjured tissues from extracellular fluid rendered hypotonic by loss of sodium to injured cells.

The prompt occurrence of circulatory failure from redistribution of extracellular water following sodium depletion[11] has been carefully studied (28).

[11] This type of shock is not complicated by the influx of K released from injured tissues.

Likewise, Gilman (29) found that sodium-depleted dogs went into collapse after minor blood losses of no consequence to normal dogs.

Since the degree of shock clinically varies with the severity, extent and nature of the injury, it is of practical value to compare the changes in the tissues and the circulation after potentially fatal and non-fatal injuries (table 6). After the potentially fatal bilateral injury the potassium released into the circulation is approximately double that extruded after the non-fatal injury to one leg. In sharp contrast the fluid accumulated in the two injured legs is not double that lost in the single injury. However, the sodium deposited in exchange (col. *h*) would make available $\frac{0.088}{0.15} = 0.58$ gram or nearly twice as much extracellular water to enter uninjured cells. Thus the obvious local loss of 0.50 gram of

TABLE 6. *Comparison of fluid loss and electrolyte changes after single and bilateral tourniquet injury*

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)
	K IN LEG	K DECREASE BELOW NORMAL LEG	H_2O IN LEG	H_2O INCREASE ABOVE NORMAL LEG	Na IN LEG	TOTAL Na INCREASE ABOVE NORMAL LEG	Na IN LOCAL EDEMA FLUID	EXCESS Na IN LEG (*f*)−(*g*)
	mEq.	*mEq.*	*grams*	*grams*	*mEq.*	*mEq.*	*0.15x(d)*	
Normal #1[1]	0.094	—	0.79	—	0.075	—	—	—
Single tourniquet leg #6[1]	0.049	0.045	1.30	0.52	1.205	0.130	0.078	0.052
Bilateral	0.059	0.035	1.07	0.28	0.156	0.081	—	—
Tourniquet legs	0.049	0.045	1.07	0.22	0.157	0.082	—	—
Sum of both legs	0.108	0.080	2.08	0.50	0.313	0.163	0.075	0.088

[1] Normal and injured mice legs from table 1; dry weights similar.

fluid (col. *d*) is associated with approximately 0.58 gram of concealed fluid loss. Their sum, 1.08 gram, is somewhat greater than the local and concealed fluid loss after single tourniquet injury: (0.52 (col. *d*) + $0.3\left(\frac{.052}{.15}\right) = 0.82$ gram). Furthermore, with more extensive injury there is less uninjured tissue to absorb the additional potassium released from the injured cells and the extracellular water dissipated by the sodium exchange. The more serious injury is not associated with a greater local loss of fluid but with greater electrolyte changes and osmotic shifts of fluid, local and systemic.

In burn shock, also, local fluid loss seemed relatively unimportant. Single legs burned at 75°C. for 5 seconds gained fluid like single-tourniquet legs and the mice survived although the burned legs became gangrenous and sloughed away. In distinct contrast, single legs burned at 95°C. for one minute gained practically no fluid, as first pointed out by Prinzmetal, Bergman and Hechter (16); nevertheless, most of these mice died soon after scalding. Prompt admin-

istration of 20% of body weight of 0.9% sodium chloride solution was without benefit; a result to be anticipated in view of the fact that local fluid and sodium losses were trivial. The rapid release of potassium from the burned tissues into the circulation may have proven fatal in many of the mice that died with convulsions similar to those seen in normal mice poisoned with potassium. In some slightly less severely burned mice that appeared in good condition 5 hours later, higher potassium values in uninjured tissues were found, suggesting that when a greater portion of the extruded potassium entered peripheral tissues, lethal concentrations in vital tissues were avoided. The sodium exchange resulted in some water shift[12] into uninjured cells after the inevitable relative hypotonicity of extracellular fluid.

Therapeutic indications. These experiments indicate two fundamental requirements for effective shock therapy: *a*) restoration of extracellular fluid volume; *b*) removal of the potassium released. Experimental (17, 18, 14) and clinical (5) investigations including plasma and urine analyses have shown that these objectives are accomplished in most instances by the prompt administration and absorption of isotonic solutions of sodium salts equal to 10–15% of body weight in the first 24 hours after injury.

It is significant that the most recent recommendations (33) of the Subcommittee on Shock of the National Research Council state "Replacement therapy in the first 48 hours thus involves fluid[13] volumes totalling 8000 to 15,000 cc." and suggest that acidosis be corrected.

SUMMARY AND CONCLUSIONS

1. Tissues injured by tourniquet trauma or by scalding at 75°C. lost potassium and gained sodium in addition to a considerable gain of extracellular fluid (water and sodium). Burns produced at 94–99°C. showed no significant local fluid accumulation but tissue-cell potassium was extruded and an equivalent intracellular gain of sodium occurred.

2. Despite considerable edema at the site of injury, the opposite uninjured leg did not lose appreciable water but lost sodium and gained potassium, pointing to extracellular dehydration with intracellular swelling.

3. Extensive local fluid loss was correlated with non-fatal injuries.

4. Death in shock was not correlated with extensive local fluid loss but with extrusion of considerable potassium from injured tissue cells and their acquisition of an equivalent amount of sodium. This exchange resulted in swelling of uninjured tissue cells throughout the body, leading to additional reduction in extracellular fluid and blood volumes.

[12] The resulting decrease in blood volume might be the cause of the vaso constriction observed by Page (31). Any increase in the osmotically active constituents of uninjured cells (Fenn, 32) would further reduce blood volume by imbibition of water from the plasma; evidence that this occurred is provided by the extremely high plasma sodium concentrations—over 168 mEq. per liter compared to the normal value of 150—found after high temperature burns and the elevated hematocrit values of 55-65 %.

[13] Recent comparative studies (37) showed that, in burn shock, solutions of albumin or other colloids or dextrose without Na were of no value and that the effect of colloids dissolved in 0.9% NaCl could be attributed to their sodium and water.

We express thanks to Dr. A. R. Dochez for his encouragement and support, to Dr. D. J. McCune for advise and assistance, and to Miss Betty B. Freeman for help with the analyses. Radioactive isotopes were prepared by the Radiation Laboratory of the Physics Department of Columbia University, which also assisted us in the counting techniques. After the termination of the OSRD contract, this work was concluded with the aid of a grant from William R. Warner and Co.

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INDEPENDENCE OF PHOSPHATE REABSORPTION AND GLOMERULAR FILTRATION IN THE DOG[1]

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Received for publication July 11, 1947

Expressing the rate of tubular reabsorption of a substance in terms of milligrams or millimols per 100 ml. of glomerular filtrate serves to bring into agreement data obtained in animals of varying sizes and renal functional capacities. A correlation between glomerular and tubular function is to be expected on morphological grounds, for, in general, tubular mass is proportional to size and numbers of glomeruli. Of more physiological interest is whether or not the capacity of the renal tubules of a given animal to reabsorb some specific substance is functionally related to the glomerular filtration rate, in the sense that a functional increase in filtration rate is accompanied by an equivalent increase in rate of reabsorption. The reabsorptive mechanisms for chloride and bicarbonate exhibit this property (1, 5, 10), that for glucose does not (11). The latter mechanism exhibits a limited transfer capacity, which in a given animal is fixed and independent of glomerular filtration rate.

Harrison and Harrison (3), and Barclay and Kenney (2) have claimed that the rate of reabsorption of phosphate is directly proportional to filtration rate. Pitts and Alexander (8, 9) have expressed the opposite view, namely that the tubular capacity to reabsorb phosphate is fixed, limited and independent of filtration rate in the same sense as is the tubular capacity to reabsorb glucose. Since none of the studies cited above has been sufficiently critical to provide an unequivocal answer to the question, we have undertaken a reinvestigation of the relation between phosphate reabsorption and glomerular filtration. Interest in this problem stems from the necessity to describe accurately the several renal mechanisms for reabsorption of electrolytes in order to understand the means by which the ionic pattern and total ionic concentration of the body fluids is established and maintained. Our results indicate that the tubular reabsorptive capacity for phosphate, like that for glucose and unlike those for chloride and bicarbonate, is fixed, limited and independent of filtration rate.

METHODS. Our experiments have been performed on two trained female dogs adequately hydrated at the start of each experiment by the administration of 500 ml. of water by stomach tube. The rate of glomerular filtration was varied by altering both the protein content of the maintenance diet and the time after the last meal at which the experiment was performed.[2] Minimum filtration rates

[1] Aided by grants from the United States Public Health Service and the John and Mary R. Markle Foundation.

[2] In preliminary experiments it was found that the rate of phosphate reabsorption was depressed by saturation of the tubular mechanism for the reabsorption of alanine or glycine. Elevation of plasma amino nitrogen from a normal value of 4 or 5 mgm. % to 25 mgm. % by

were obtained with the animal in a postabsorptive state (12 to 14 hours after feeding) following maintenance on a cracker-meal diet for 2 weeks or more. Intermediate values were obtained with the animal maintained on a dog chow diet or on a ground beef-heart diet. The highest filtration rates were obtained in animals 3 hours after a meat meal (6). Plasma phosphate concentration was elevated to 3.5 to 5.5 millimols per liter by the intravenous infusion of neutral (pH 7.4) sodium phosphate. At such plasma levels the phosphate load presented

TABLE 1. *Experiments on dog 1 which illustrate the independence of the absolute quantity of phosphate reabsorbed by the renal tubules and the rate of glomerular filtration*

Filtration rate was altered by varying the protein content of the maintenance diet and the time following the last meal

URINE FLOW	GLOMERULAR FILTRATION RATE	PHOSPHATE				
		Plasma concentration	Filtered	Excreted	Reabsorbed	
			Fasting—carbohydrate diet			
ml/min.	*ml/min.*	*mM/L*	*mM/min.*	*mM/min.*	*mM/min.*	*mM/100 ml. filtrate*
3.4	64.2	4.55	0.292	0.159	0.133	0.207
3.1	68.7	4.88	0.335	0.206	0.129	0.188
2.8	68.7	5.22	0.359	0.231	0.128	0.187
2.8	68.6	5.55	0.381	0.251	0.130	0.190
			Fasting—meat diet			
9.1	80.0	4.55	0.364	0.237	0.127	0.159
9.7	83.0	4.70	0.390	0.265	0.125	0.151
10.8	85.3	4.84	0.413	0.290	0.123	0.144
10.3	80.0	4.95	0.396	0.272	0.124	0.155
			Absorptive—meat diet			
10.0	105	4.39	0.461	0.335	0.126	0.120
7.9	107	4.60	0.492	0.372	0.120	0.112
7.0	108	4.73	0.511	0.371	0.140	0.130
9.0	105	4.70	0.495	0.365	0.130	0.124

to the tubules in the glomerular filtrate exceeds by some 2.5 to 5 times their capacity to reabsorb. Hence saturation of the tubular mechanism is assured and the rate of phosphate reabsorption (Tm PO_4) becomes a valid measure of one

the infusion of alanine resulted in a depression of phosphate reabsorption to about 35% of its control value. Thus, acute experiments in which glomerular filtration rate was elevated by the infusion of amino acids were not deemed feasible. It was found that there was no significant difference between the fasting plasma amino nitrogen concentrations of animals maintained on a carbohydrate and on a meat diet. Even 3 hours after a meal of 2 pounds of ground beef heart there was a rise of only 1.5 mgm. % in the plasma amino nitrogen. It was felt that such minor increases in plasma amino nitrogen would be without any significant effect on phosphate reabsorption.

discrete renal function. The creatinine clearance has been used as a measure of glomerular filtration rate. Chemical methods and calculations have been described in a previous communication (9).

RESULTS. Three representative experiments on *dog 1* are presented in table 1. When the animal was studied in a postabsorptive state during maintenance on a carbohydrate diet, the glomerular filtration rate averaged 67.5 ml. per minute; on a meat diet, 82.1 ml. per minute; and three hours following a large meat meal, 106 ml. per minute. At plasma concentrations between 4.39 and 5.55 millimols

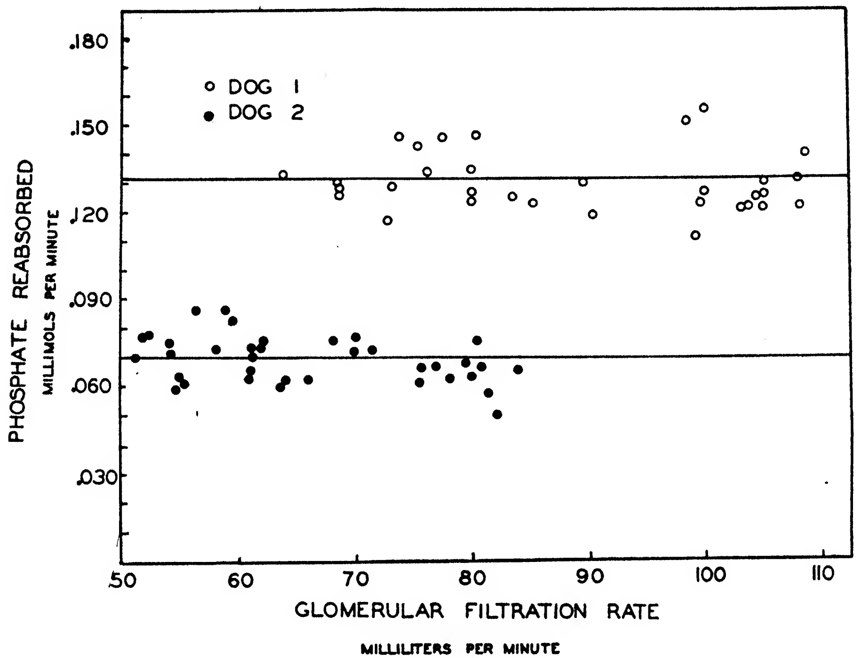

FIG. 1. Experiments illustrating the independence of the absolute quantity of phosphate reabsorbed (expressed in millimols per minute) and the rate of glomerular filtration. Data from 18 experiments on *dogs 1* and *2*.

per liter the quantity of phosphate filtered through the glomeruli far exceeded the quantity which the tubules could reabsorb. Consequently, large quantities of phosphate were excreted in the urine. In the last two columns of table 1, the quantity of phosphate reabsorbed is presented in two ways: first, in absolute terms of millimols reabsorbed per minute, calculated as the difference between the quantity filtered and the quantity excreted; and second, in terms of millimols per 100 ml. of glomerular filtrate, calculated as the dividend of the absolute amount reabsorbed by the filtration rate times 100. It is apparent that the absolute amount of phosphate reabsorbed is constant within limits of 0.120 and 0.140 millimols per minute despite large changes in filtration rate. Such variations as

are observed in this series are well within the range of experimental error. In contrast, the quantity of phosphate reabsorbed per 100 ml. of glomerular filtrate is far from constant, ranging from 0.112 to 0.207 millimols and varies as an inverse linear function of glomerular filtration rate.

In all, 18 similar experiments were performed on 2 dogs. The absolute quantity of phosphate reabsorbed is plotted in figure 1 against glomerular filtration rate. In *dog 1* the mean rate of reabsorption was 0.132 millimols per minute; in *dog 2*, 0.070 millimols per minute. In neither animal is there evidence of any consistent variation in the quantity of phosphate reabsorbed with glomerular filtration rate, although the latter was varied over a range of nearly 100% in each of the 2 animals. Rather, rate of reabsorption remains constant within reasonable limits of experimental variation at a value which is characteristic for a given animal. Thus, calculations of the quantity of phosphate reabsorbed per 100 ml. of glomerular filtrate have no functional significance in the normal dog.

DISCUSSION. It is generally conceded that in the normal mammalian kidney all glomeruli are functioning at all times (11, 14). Changes in filtration rate such as those produced by altering the protein content of the diet are brought about by changes in afferent and efferent anteriolar tone, whereby the effective intracapillary filtration pressure is altered (7). An increase in filtration rate does not, therefore, result from the opening up of previously quiescent glomeruli, but rather from an increase in the quantity of filtrate formed in each glomerulus. The entire tubular reabsorptive mass is normally functional at all times.

The renal tubular mechanisms for the reabsorption of glucose and phosphate may be saturated in their entireties by sufficient elevations of their respective plasma concentrations, even at the low glomerular filtration rates observed when protein intake is restricted. When saturated the tubular cells transfer these solutes at maximal rates, and an increase in filtration rate is without effect on reabsorption.

The mechanisms responsible for the transfer of chloride and bicarbonate are fundamentally different, for the reabsorption of these ions is related to the reabsorption of water. Some 80 to 90% of the fluid filtered at the glomeruli is reabsorbed in the proximal tubules (12), and this reabsorption appears to be essentially isosmotic (13). Since sodium, chloride and bicarbonate constitute the majority of the osmotically active constituents of the filtrate, it follows that these ions must be reabsorbed in the proximal tubule more or less in direct proportion to the reabsorption of water. It has been previously shown that the quantity of each of these ions reabsorbed per unit of time is directly related to the glomerular filtration rate (5, 10); obviously the quantity of water reabsorbed is likewise related to filtration rate. 1) Does the active reabsorption in the proximal tubule of some ion such as sodium determine the reabsorption of water, and do certain other ions such as chloride and bicarbonate (but not phosphate) follow passively by diffusion; 2) does the active reabsorption of water in the proximal tubule condition the back diffusion of the three major ionic constituents; or 3) does the colloid osmotic force resident in the concentrated plasma proteins of the peritubular capillaries determine the diffusion of both water and ions through the

epithelium of the proximal tubules? There is at present no conclusive evidence to support any of these possible views. If, however, the proximal tubules were relatively permeable to water and to sodium, bicarbonate and chloride ions, the colloid osmotic pressure of the plasma proteins could cause the return of fluid to the peritubular capillaries. Furthermore, the greater the rate of formation of filtrate, the greater would be the force available for the return of fluid. Thus the rate of reabsorption of chloride, bicarbonate and sodium would be proportional to the rate of formation of glomerular filtrate. In addition to this proximal tubular mechanism dominated by the physical force of colloid osmotic pressure, there must obviously be active types of distal tubular reabsorptive mechanisms capable of preferential reabsorption of ionic constituents and even of nearly complete removal of certain of them from the final urine.

Evidence at present available indicates that the reabsorptive mechanism for sulfate is similar in its properties to that for phosphate although distinct from it (4). It is therefore apparent that there are no less than two broad general categories of reabsorptive mechanisms for electrolytes; namely, those in which rate of transfer is fixed, limited and independent of glomerular filtration rate, e.g., phosphate and sulfate; and those in which the rate of transfer is proportional to glomerular filtration rate, e.g. sodium, chloride and bicarbonate. Generalizations such as those of Barclay and Kenney (2) based on an assumed qualitative similarity of the reabsorptive mechanisms for all ions are therefore erroneous.

There is the possibility that circumstances could be found under which the absolute reabsorption of phosphate might vary in proportion to the rate of glomerular filtration. If filtration rate were reduced by the complete closure of some glomeruli, the reabsorptive capacity exhibited by their attached tubules would be lost. Such closure of glomeruli might be expected to occur in shock and in severe dehydration. It is possible that some of the discrepancies between the results presented above and those described by Harrison and Harrison (3) might be explained on this basis, for they reduced filtration rate by dehydration induced by hypertonic peritoneal lavage and by the use of diuretics.

It seems scarcely necessary to point out that when errors are introduced into the determination of filtration rate as a result of failure to collect completely the urine formed within a specified time interval, an equal error will be introduced into the determination of reabsorptive capacity for phosphate. Correcting such results to any standard filtration rate will obviously reduce variability. Certain other discrepancies between published work and our own results may be explained on this basis.

SUMMARY

In a series of 18 experiments performed on 2 dogs the rate of tubular reabsorption of phosphate was assessed at sufficiently high loads to assure saturation of the reabsorptive mechanism. It was found that rate of phosphate reabsorption expressed in millimols per minute was constant for a given animal and independent of glomerular filtration rate when that rate varied over a wide range by altering the protein content of the diet.

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EFFECT OF PITRESSIN ON THE URINARY EXCRETION OF CHLORIDE AND WATER IN THE HUMAN[1]

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Received for publication July 15, 1947

There is general agreement that the posterior pituitary gland is concerned with the excretion of water by the kidney. It has been a universal experience that the urinary chloride concentration is increased during the antidiuretic action of the hormones of the posterior pituitary, but the experience regarding the rate of chloride excretion during the antidiuresis has been more variable.

Reports on the effect of whole posterior pituitary extracts in rats (1, 2, 3) and in dogs (4, 5) agree that the rate of chloride excretion is increased. It has been reported that pitressin increased the rate of chloride excretion in dogs (6). A transient early increase in the chloride excretion rate in dogs occurred during the repeated administration of the more slowly absorbed pitressin tannate in oil (7). On the other hand, in two patients with diabetes insipidus the excretion of sodium and chloride was less during pitressin therapy than during a period without treatment (8).

Some doubt has arisen as to whether or not the increased rate of excretion of chloride following the administration of posterior pituitary extracts can be attributed to the antidiuretic hormone itself. Fraser (9) found that an oxytocic principle was much more effective in increasing the rate of chloride excretion in non-hydrated rats and dogs than was a pressor principle, and Dicker and Heller (10) found that in rats small amounts of pitocin or pituitrin produced a significant increase in the rate of chloride excretion, while pitressin was without effect. Hare, Hare, and Phillips (11) reported that pitressin had no effect upon the tubular reabsorption of chloride in dogs. Ralli *et al.* (12) reported that pitressin lost its chloruretic activity during dialysis while it retained its antidiuretic activity.

Another difference of opinion concerns the question as to whether the posterior pituitary hormones have a specific effect upon the tubular reabsorption of chloride, or whether the effect is incidental to other changes such as increased water reabsorption or increased glomerular filtration. Silvette (2) concluded that in the rat a posterior-pituitary extract had an effect directly upon the reabsorption of chloride by the tubule. In dogs Shannon (5) found that a posterior-pituitary extract caused an increased excretion of sodium and chloride in 3 of 4 dogs with experimental diabetes insipidus. There was no change in the rate of glomerular filtration with the relatively low dosage used, and he concluded that

[1] Presented in part before the American Physiological Society, Chicago, 1947. Fed. Proc. 6: 222, 1947.

there was a depression of chloride reabsorption by the tubules. Hare, Hare, and Phillips (11), however, concluded that in the dog with diabetes insipidus, pitressin (0.1–2.0 mU) produced a marked decrease in urine flow without an increase in chloride excretion. In a review of the problem of water excretion, Smith (13) states that the chloruretic action of posterior-pituitary hormones is possibly not a specific effect upon chloride reabsorption in the tubules but may be referable to a change in the glomerular filtration rate.

We have studied the effect of pitressin upon the urinary water and chloride excretion and the urinary chloride concentration in hydrated and non-hydrated human subjects.

PROCEDURE. Twenty-eight patients, who were hospitalized for diagnostic study, were used as subjects. None of these showed any evidence of endocrine dysfunction, nor of disturbances of fluid or acid-base balance. A test of the concentrating capacity of the kidneys in these subjects revealed only one in whom there was evidence of considerable renal functional impairment.

First day. Starting at 9 A.M. the subjects drank 200 cc. of tap water every 30 minutes until 5 P.M. to insure hydration. Lunch, consisting of the house diet, was taken as usual. At 2 P.M. the subject voided and the specimen was discarded. At 3 P.M. the subject voided as completely as possible, and this specimen was saved and analyzed as the control specimen. Immediately after voiding, the subject received 10 units of pitressin (Parke, Davis & Co.) intramuscularly. At 4 and 5 P.M. the subject again voided completely, and these specimens were saved for analysis.

Second day. At 2 P.M. on the following day the procedure was repeated without special hydration procedure, water being taken *ad lib.* Blood was drawn at 3 P.M. for determination of the serum chloride concentration. The serum chloride concentrations were determined according to the procedure of Wilson and Ball (14), and the urinary chloride concentrations were determined according to a modified Volhard-Harvey procedure (15). The rate of chloride excretion, expressed as mgm. NaCl per hour, was calculated from the urine volume and the chloride concentration.

In 4 subjects water diuresis was produced by drinking 200 cc. of tap water every 30 minutes for 3 hours. The urinary chloride concentration and excretion rate was determined on hourly voided urine samples.

RESULTS. *The relationship between the serum chloride concentration and the excretion of chloride.* In order to determine if the serum chloride concentration affected our results, the significance of the difference between the mean values obtained for urinary chloride concentration and excretion rate in the subjects in which the serum chloride concentration was less than 98 mEq/l (mean = 93) and the mean values in the subjects in which the serum chloride concentration was greater than 100 mEq/l (mean = 103) was compared statistically using Fisher's t test. For this purpose the data were also divided into hydrated and non-hydrated groups.

The difference between the means, in the above groups of subjects, for none of the following measurements was significant at the 5% level: the control chloride

concentration, the control chloride excretion rate, and the chloride excretion rates for the first and second post-pitressin hours.

The relationship between the hydration state and the excretion of water and chloride. In order to determine if the state of hydration affected chloride excretion, the significance of the differences between the mean values obtained for the rate of water excretion and the urinary chloride concentration and excretion rate were examined statistically in the hydrated versus the non-hydrated groups. The results are found in table 1. As one would expect, the control rate of water excretion was significantly greater in the hydrated subjects. The control urinary chloride concentration was significantly greater in the non-hydrated group. The rate of water excretion during the first post-pitressin hour was significantly greater in the hydrated than in the non-hydrated subjects, but the chloride excretion rates in the post-pitressin hours did not differ significantly between the hydrated and non-hydrated groups.

TABLE 1. *Relation between hydration state and various measurements. Chloride concentration expressed as m.Eq./l., chloride excretion as mgm./hr. NaCl, water excretion as cc/hr. H = hydrated, NH = non-hydrated*

	CONTROL WATER EXCRETION		CONTROL CHLORIDE CONCENTRATION		CONTROL CHLORIDE EXCRETION		WATER EXCRETION HOUR 1		WATER EXCRETION HOUR 2		CHLORIDE EXCRETION HOUR 1		CHLORIDE EXCRETION HOUR 2	
	H	NH	H	NH	H	NH	H	NH	H	NH	H	NH	H	NH
Mean	188.3	119.9	24.3	38.1	240	237	120.2	61.7	60.8	48.8	243	225	237	229
σ	96.1	77.5	20.6	26.6	249	167	79.9	36.1	37.7	34.2	118	164	163	177
n	26	26	26	26	26	26	26	26	25	25	26	26	26	25
t	2.83		2.09		0.06		3.40		1.18		0.41		0.18	
P	<0.01		<0.05		>0.05		<0.01		>0.05		>0.05		>0.05	

The effect of pitressin on the urinary chloride concentration when the rate of water excretion was not decreased. In 18 experiments on 16 subjects (table 2) there was either no significant change in the rate of water excretion during the first post-pitressin hour or the rate was increased. In 15 of these experiments the urinary chloride concentration was increased during this hour. The rate of chloride excretion was also increased. In 3 experiments both the chloride concentration and the rate of excretion decreased during the first post-pitressin hour.

The effect of water diuresis on the urinary chloride concentration. In 5 experiments on 4 subjects (table 3) the urinary chloride concentration decreased progressively during water diuresis. The rate of chloride excretion was somewhat erratic during the diuresis.

The effect of pitressin on the urinary rate of chloride excretion when there was a decreased rate of water excretion. In 47 of the total of 65 experiments (table 4) the rate of water excretion decreased during both the first and second post-pitressin hours. With two exceptions, the urinary chloride concentration in these subjects increased. The effect of pitressin on the rate of chloride excretion, however, was

variable. When compared with the control rate, the rate of chloride excretion decreased during the first post-pitressin hour in 25 of 37 experiments and during

TABLE 2. *The effect of 10 units pitressin on chloride excretion when there was little change or an increase in water excretion*

SUBJECT NUMBER	URINE VOLUME CC./HR.		URINE CHLORIDE CONCENTRATION MEQ/L			TOTAL CHLORIDE EXCRETION MGM./HR.		
	Control Hour	Post-Pitressin Hour 1	Control Hour	Post-Pitressin Hour 1	% Change	Control Hour	Post-Pitressin Hour 1	% Change
				Non-hydrated				
(1)	60	62	13.60	48.96	+258	48	193	+302
(2)	30	25	44.20	105.40	+139	78	155	+98
(3)	20	24	28.56	36.72	+28	34	52	+53
(4)	50	69	21.08	92.48	+339	62	375	+505
(5)	15	67	17.00	36.04	+112	15	142	+847
(6)	26	110	86.36	117.64	+36	132	761	+477
(7)	147	173	36.72	44.20	+21	318	450	+42
				Hydrated				
(7)	224	219	21.76	33.32	+53	287	429	+49
(7)	42	372	23.80	40.12	+69	59	878	+1390
(8)	180	195	31.28	18.56	−41	331	211	−36
(9)	184	200	16.32	27.20	+67	177	320	+81
(10)	172	215	25.16	42.84	+70	255	542	+112
(11)	38	126	7.48	13.60	+82	17	101	+495
(12)	52	75	19.72	50.32	+170	60	222	+270
(13)	25	120	14.96	23.12	+54	22	163	+640
(14)	178	205	13.60	10.20	−25	142	123	−14
(15)	248	285	48.96	15.64	−68	714	262	−63
(16)	120	220	6.80	34.00	+400	48	440	+816

[1] Expressed as NaCl.

TABLE 3. *Chloride excretion during water diuresis*

SUBJECT	HOUR 1			HOUR 2			HOUR 3		
	Volume	Concn.	Excretion	Volume	Concn.	Excretion	Volume	Concn.	Excretion
	cc.	*mEq/l*	*mgm/hr*	*cc.*	*mEq/l*	*mgm/hr*	*cc.*	*mEq/l*	*mgm/hr*
(H)	30	210.0	368	80	195.0	905	75	132.4	576
(W)	56	229.1	744	126	103.5	756	398	22.2	513
(W)	45	126.4	330	213	29.1	359	354	14.9	305
(S)	122	12.0	85	265	1.7	26			
(R)	412	8.6	306	380	7.7	169	207	6.0	72

the second hour in 30 of 46 experiments. In the remainder of the experiments the rate of chloride excretion increased. The discrepancy in the total number of experiments between the data for the first and second post-pitressin hours is due

Table 4. *The effect of 10 units pitressin on urinary chloride concentration and excretion when there was a decrease in water excretion*

Subject No.	Urine volume cc./hr.			Chloride concentration meq./l.			Total chloride excretion mgm./hr.		
	Control Hr.	Hr. 1	Hr. 2	Control Hr.	Hr. 1	Hr. 2	Control Hr.	Hr. 1	Hr. 2
				Non-hydrated					
1	60		15	13.60		69.36	48		61
6	202	164	65	36.72	42.16	88.40	436	407	338
7	147		31	36.72		93.16	318		170
7	91	50	29	59.16	99.28	129.20	317	292	220
8	283	65	67	19.72	51.00	51.00	528	195	201
9	220	80	145	12.92	12.24	21.76	167	58	186
10	221	76	55	25.16	42.84	95.20	255	542	336
11	164	148		17.00	47.60		164	414	
13	70	47	60	40.80	74.12	85.00	168	218	300
14	72	48	22	108.80	58.48	99.28	461	165	128
15	175	75	37	36.04	50.32	94.52	371	222	206
16	160	90	24	30.60	66.64	125.80	288	353	178
17	217	79	108	37.40	44.80	85.68	477	209	544
18	140	39	34	42.50	129.88	140.80	350	298	286
19	215	17	40	19.04	62.56	49.64	241	63	117
20	80	25	16	9.52	28.60	28.60	45	42	27
21	43	28	15	24.48	36.04	38.76	62	59	34
22	192	32	31	30.60	52.36	51.00	346	96	93
22	182	60	68	46.24	108.12	61.88	495	382	248
23	98	49	36	17.68	18.36	26.52	102	53	56
24	59	44	22	66.64	65.96	125.80	231	171	163
25	92	47	27	101.32	155.04	165.24	548	429	262
26	150	37	28	51.00	70.04	105.40	450	152	174
				Hydrated					
1	385	225	52	10.20	29.27	85.40	231	387	260
2	105	60	48	73.76	90.44	126.48	449	319	357
3	178	94	61	1.36	10.88	20.40	14	60	73
4	222	37	40	8.84	108.12	93.50	115	235	220
5	260	143	103	12.24	40.12	90.00	187	337	544
6	227	94	69	58.48	90.44	119.26	781	500	486
6	120		42	23.12		29.58	163		73
7	224		27	21.76		136.00	287		216
8	180		60	31.28		61.88	331		218
9	184		65	16.32		110.80	177		424
10	172		60	25.16		95.20	255		336
12	52		38	19.72		48.28	60		108
14	178		60	13.60		22.44	142		79
15	248		53	48.96		79.56	714		247
17	79	37	60	57.12	76.84	86.36	265	167	305
18	83	18	20	29.24	46.24	27.88	143	49	83
19	214	23	21	16.66	52.36	78.88	210	71	97
20	162	18	103	2.04	31.28	8.16	19	33	49
21	283	27	19	20.40	45.56	98.60	340	72	110
22	215	139	47	27.88	59.16	84.32	353	484	238
23	181	42	24	14.96	23.86	27.80	159	59	39
24	320	24	39	22.44	144.84	78.20	422	204	179
26	420	205	60	6.80	21.08	62.56	168	254	221
27	202	160	83	12.24	43.52	70.72	145	410	345

to the inclusion of experiments in the latter when the rate of water excretion did not decrease in the first hour (table 2) but did decrease in the second hour.

DISCUSSION. *The relationship between the serum chloride concentration and the hydration state and the excretion of water and chloride.* The statistical analysis of the data for the relationship between the serum chloride concentration and the excretion of chloride clearly indicated that the control chloride concentration, the control chloride excretion rate and the effect of pitressin on the chloride excretion rate are not related to the serum chloride concentration.

The control urinary chloride concentration was greater in the non-hydrated than in the hydrated subjects. Since it has been demonstrated that in the adult human being the glomerular filtration rate is not specifically related to hydration (19), the difference must represent a decreased water reabsorption or a greater tubular chloride reabsorption or the resultant of both factors in the hydrated group.

The fact that the rate of water excretion during the first post-pitressin hour was significantly greater in the hydrated than in the non-hydrated subjects while the rate of chloride excretion during the post-pitressin hours was not significantly different between the two groups of subjects suggests that the chloride excretion rate following pitressin may vary independently of the water excretion rate, and that separate control factors may be involved.

An increase in urinary chloride concentration when the rate of water excretion was not decreased by pitressin. The data in table 2 show that in this group of subjects the urinary chloride concentration increased. The usual explanation for the increased urinary chloride concentration following pitressin is that water reabsorption is greatly increased by pitressin, and the increased urinary chloride concentration results because of a lesser reabsorption rate. However, in these experiments the water excretion was either unchanged or it was increased; so the increased chloride concentration cannot be explained by preferential water reabsorption.

Assuming that if the rate of glomerular filtration of chloride were unchanged by pitressin, an increased urinary chloride concentration might occur if there were a decrease in the rate of diffusion of chloride from the tubular fluid across the epithelium to the blood. A decrease in chloride diffusion might occur if the diffusion gradient between tubular fluid and the plasma were decreased. This could occur if the plasma chloride concentration were increased by pitressin, which is certainly unlikely in these experiments. A decrease in chloride diffusion might also occur if the duration of the exposure to the reabsorbing epithelium were diminished due to a more rapid flow through the portion of the tubule concerned with chloride reabsorption. Such a situation might conceivably exist in these experiments, especially in the hydrated subjects, if pitressin absorption had not occurred, and if one were dealing with water diuresis alone. However, it will be seen in table 3 that during water diuresis in 4 subjects (5 experiments) there was a consistent decrease in urinary chloride concentration. Therefore, it does not seem possible to explain the observed increase in urinary chloride concentration following pitressin in these experiments on the basis of decreased diffusion.

Another factor which might increase the urinary concentration of chloride must be considered. If pitressin increased the rate of glomerular filtration, and if the proximal tubule reabsorbed some constant fraction of the filtrate water without reabsorbing chloride to the same extent, then it is conceivable that an increased concentration of chloride would be presented to the more distal portions of the tubule, and thus chloride might appear in the bladder urine in a higher concentration. The results of studies of the effect of posterior-pituitary preparations on glomerular filtration rate are quite variable. Walker *et al.* (17) report that in 11 dogs receiving 0.5 cc. of pituitrin subcutaneously, 6 showed an increased glomerular filtration and 5 showed a decreased filtration rate. Dicker and Heller (10) reported that in rats 0.3 mU/100 grams of body weight of pitressin resulted in a significant increase in the glomerular filtration rate whereas 3.0 mU/100 grams resulted in no change in the mean glomerular filtration rate, although some individuals showed either a significant increase or decrease. Hare *et al.* (11) reported that pitressin did not affect the glomerular filtration rate in a dog with diabetes insipidus. Burgess, Harvey, and Marshall (18) reported that in one human subject there was an initial slight decrease in the glomerular filtration rate following 0.1 U/kgm. of pitressin, which was followed by a slight increase; another subject showed only an increase (17–21%). In 2 dogs 0.5 and 0.2 U/kgm. of pitressin resulted in a slightly decreased glomerular filtration rate in both instances. It appears that the effect of pitressin on the glomerular filtration rate is quite variable and is not very marked.

As to what effect variations in glomerular filtration rate may have upon the urinary chloride concentration, Walker *et al.* (16) have reported that in the guinea-pig and rat the proximal tubule reabsorbs isosmotically a constant fraction of the glomerular filtrate, but that the tubular fluid-plasma ratio of chloride concentration increases so that the average value is 1.4. This is presumably due to a selective rejection of chloride in the proximal tubule. Upon inspection of their data it was noted that there was no relation between the rate of flow of fluid in the proximal tubule and the ratio of chloride concentration. This is particularly true in the more distal portions of the first third of the proximal tubule. To quote from their paper: "The most logical explanation of these events is that chloride appears in glomerular fluid in concentrations appropriate to an ultrafiltrate of blood plasma, is concentrated in the early proximal tubule by the reabsorption of a nearly chloride-free fluid, and is maintained at this concentration by the reabsorption in the later proximal tubule of a fluid containing approximately 1.4 times the chloride concentration of plasma." It thus appears that if the glomerular filtration rate increases, a slightly increased volume of fluid with a rather constant chloride concentration is presented to the more distal portions of the tubule. Variations in chloride concentration then must be accounted for by variations in water and chloride reabsorption in the distal portions of the tubule. Lotspeich, Swan and Pitts (20) report that functional changes in the rate of glomerular filtration in the dog are correlated with direct and nearly proportional changes in the capacity of the renal tubules to reabsorb chloride. It

then seems very unlikely that even if pitressin should increase the rate of glomerular filtration, this could account for the increased chloride concentration observed in these experiments.

We are then left with the possibility that pitressin, or some other hormone present in the preparation, exerts a specific inhibitory effect upon the mechanism responsible for the reabsorption of chloride in the distal portions of the tubule, thus increasing the concentration of chloride in the tubular fluid and in the bladder urine. Such a mechanism will explain the above observations.

A decreased urinary chloride concentration and excretion rate following pitressin. In 3 experiments (table 2, nos. 8, 14, 15) a decrease in the chloride concentration and in the total chloride excretion occurred during the first post-pitressin hour. Each of these was in a hydrated individual with an established water diuresis, and in each of these the urinary chloride concentration exceeded the control during the second post-pitressin hour (table 4). These undoubtedly, therefore, represent instances in which there was a delayed absorption of pitressin.

The increased excretion of chloride in all of the other experiments is a natural result of the changes in urine volume and chloride concentration.

Increased excretion of water following pitressin. An increased excretion of water was seen in 16 experiments after pitressin. There are two possible explanations for this increased water excretion during the first post-pitressin hour.

The increased chloride concentration resulting from an inhibition of tubular reabsorption of chloride might cause an osmotic diuresis by interfering with the reabsorption of water in the distal tubule. In two instances a further increase in the water excretion rate occurred during the second post-pitressin hour, and in one of these the chloride concentration was unchanged while in the other one the chloride concentration increased further. These two instances could be explained easily on the basis of an osmotic diuresis. However, in 12 instances there was a decrease in the water excretion during the second hour in spite of a further increase in chloride concentration. In 7 of these experiments the water excretion during the first hour was increased over the control, and it does not seem logical that this diuresis can be explained as an osmotic one since there was a subsequent decrease in water excretion with a further increase in chloride concentration. However, it is possible that an osmotic diuresis during the first hour was overcome by the continuing action of pitressin on water reabsorption in the second hour.

A second possibility, which will explain the above 7 experiments, is that a difference in the temporal effect of pitressin on the two mechanisms is involved, i.e., the inhibition of chloride reabsorption occurred before the increase in water reabsorption; in which case the increased water excretion during the first post-pitressin hour is merely a manifestation of delayed acceleration of water diuresis.

The decreased excretion of chloride when the rate of water excretion was decreased by pitressin. In the remaining experiments (table 4) the excretion of water decreased during both the first and second post-pitressin hours, and with two exceptions the urinary chloride concentration increased. The effect of pitressin

on the rate of excretion of chloride was variable, however; during the first hour in 25 of 37 experiments and during the second hour in 30 of 46 experiments there were decreases in the chloride excretion when compared with the control.

If pitressin has a specific inhibitory effect on chloride reabsorption some additional mechanism must be operating in the experiments in which a decreased chloride excretion rate was found. It was noted that there appeared to be a relationship between the control rate of chloride excretion and the effect of pitressin. There were 15 experiments with a decreased chloride excretion rate and 1 with an increased rate during the first post-pitressin hours, when the control chloride excretion rate was greater than 275 mgm. NaCl/hr., and 10 experiments with a decreased and 11 experiments with an increased rate when the control chloride excretion rate was less than 275 mgm. NaCl/hr. The χ^2 test indicates that this relationship is highly significant ($\chi^2 = 8.82$, df $= 1$, P $= <0.01$).[2] There were 20 experiments with a decreased and 1 with an increased chloride excretion rate during the second post-pitressin hour when the control rate was greater than 275 mgm. NaCl/hr., and 10 experiments with a decreased and 15 with an increased chloride excretion rate when the control rate was less than 275 mgm. NaCl/hr. This relationship is also highly significant ($\chi^2 = 15.35$, df $= 1$, P $= <0.01$). It is concluded that the greater the initial rate of chloride excretion the lesser the excretion of chlorides, as NaCl, following pitressin and vice versa. In these experiments an initial rate of 275 mgm. NaCl/hr. represents a critical range of initial excretion as far as the effect of pitressin is concerned.

Since the rate of excretion of chloride depends upon the chloride concentration and the urinary volume, these factors were examined statistically. It was noted that when the urinary chloride concentration in the control period was greater than 13 mEq/l, 23 experiments in the first hour and 28 in the second hour showed a decreased chloride excretion rate following pitressin while 5 in the first and 8 in the second hour showed an increased excretion rate. When the urinary chloride concentration in the control period was less than 13 mEq/l, 2 experiments in the first hour and 1 experiment in the second hour showed a decreased excretion rate following pitressin while 7 experiments in the first hour and 8 experiments in the second hour showed an increased chloride excretion rate. These relationships are highly significant (1st hour: $\chi^2 = 11.16$, df $= 1$, P $= <0.01$; 2nd hour: $\chi^2 = 13.96$, df $= 1$, P $= <0.01$). It is concluded that the lower the control chloride concentration the greater the excretion rate of chloride following pitressin, and vice versa. In these experiments an initial chloride concentration of 13 mEq/l represents a critical range of concentration as far as the effect of pitressin is concerned.

It was found that during the first post-pitressin hour the correlation between the decrease in water excretion and the decrease in chloride excretion was significant. (r $= 0.256$, df $= 72$, P $= <0.05$). During the second post-pitressin hour this correlation was not significant (r $= 0.105$, df $= 70$, P $= >0.05$), probably due to the considerable variation in this period. It is concluded that in these

[2] df denotes degrees of freedom. P denotes probability of chance occurrence.

experiments, at least during the first post-pitressin hour, the greater the reabsorption of water by the tubules the greater is the reabsorption of chloride by the tubules.

Is there an obligatory reabsorption of chloride when the rate of water excretion is decreased by pitressin? Walker *et al.* (16) have shown that in the proximal tubule there is a considerable reabsorption of water without an equivalent reabsorption of chloride, so that the chloride concentration of the tubular fluid reaches an average F/P $\frac{\text{fluid concentration}}{\text{(plasma concentration)}}$ ratio of 1.4 while the ratio for the bladder urine is less than 1.0. Since the F/P ratio of bladder urine is less than 1.0 there must have been a reabsorption of chloride in excess of water in the distal tubule. It seems probable to us that the site of this chloride reabsorption in the distal tubule is located proximal to the site of water reabsorption. The decreased excretion rate for chloride following pitressin and accompanying a decreased water-excretion rate could be explained on the basis of an obligatory chloride reabsorption in the more distal portions of the tubule along with the pitressin-induced reabsorption of water. Thus, when the control urinary chloride concentration and excretion rate is high, presumably due to a high chloruretic effect of the endogenous pituitary secretions, the addition of pitressin will have little or no further effect on the specific reabsorption of chloride, but it will induce a reabsorption of water which may be accompanied by an obligatory chloride reabsorption. An obligatory chloride reabsorption, under these circumstances, would not invalidate the thesis that pitressin, or some other hormone in the extract, has a specific inhibitory effect on the tubular reabsorption of chloride in the more proximal portion of the tubule.

Application to other observations. This concept of the control of chloride excretion by some pituitary factor is useful in explaining some previous observations on chloride excretion under a variety of circumstances. Eggleton (21) and Barclay *et al.* (22) report that during water diuresis there is usually a decrease in chloride excretion rate which is not well correlated with the increase in water output. During water diuresis there is a decrease in the excretion of the anti-diuretic hormone resulting in the water excretion, and there may also be a decrease in the formation of a posterior pituitary chloride factor resulting in an increase in the reabsorption of chloride with a decreased rate of chloride excretion. They report that during exercise the water diuresis is usually inhibited and that the chloride excretion rate is also greatly diminished. Presumably the effect of exercise on water diuresis is related to an increased secretion of the anti-diuretic hormone. The further decrease in chloride excretion may be due to a failure of exercise to produce the factor controlling chloride reabsorption, or to the obligatory reabsorption of chloride if the chloride factor were secreted in greater amounts. Eggleton and Smith (23) report that ethyl alcohol results in a marked diminution in chloride excretion even if the degree of diuresis is very slight. This can be explained on the basis of a decreased production of the factor inhibiting the tubular reabsorption of chloride without a concomitant decrease in the anti-diuretic factor.

Farnsworth (24) reported that patients with essential arterial hypertension demonstrated a specific failure to reabsorb chloride to the same extent as that found in normal subjects. She suggested that one of the explanations for this defect might be an excess secretion of pitocin. It is possible that in this condition there may be an excessive secretion of the chloride reabsorptive inhibiting factor.

The importance of the secretions of the adrenal cortex in regulating the excretion of sodium and potassium has been stressed for many years. Evidence has been presented here which supports the hypothesis that the posterior pituitary gland may also be concerned with this regulation and that the excretion of these ions is controlled by an interplay of these antagonistic factors.

SUMMARY

The effect of the intramuscular injection of 10 units of pitressin on the urinary chloride concentration and excretion rate and on the water excretion rate has been studied in 28 human subjects. These subjects were first hydrated by the oral administration of tap water (200 cc. every 30 minutes) for 5 hours before the experiment and throughout the experiment. A control urine specimen, representing one hour of excretion, was collected, the pitressin was injected, and two hourly urine specimens were collected. The experiment was repeated the following day without the special hydration procedure.

The rate of chloride excretion following pitressin was related neither to the pre-injection serum chloride concentration nor to the state of hydration of the subject.

The rate of water excretion during the first post-pitressin hour was related directly to the state of hydration. The rate of chloride excretion following pitressin was inversely related to the pre-injection rate of chloride excretion and chloride concentration. The critical control urinary levels were approximately 275 mgm. NaCl/hr. and 13.0 mEq. Cl/l.

In 15 experiments pitressin resulted in an increased urinary chloride concentration during the first post-pitressin hour in spite of an increase in the urinary water excretion rate. This is interpreted as indicating a specific inhibition of chloride reabsorption in the renal tubule by some factor present in the pituitary extract.

In 47 experiments, the injection of pitressin was followed by a decrease in the rate of water excretion which was accompanied by either an increase or a decrease in the rate of chloride excretion. The decreased rate of chloride excretion is attributed to an obligatory chloride reabsorption due to the marked increase in the rate of water reabsorption by the renal tubule.

It is suggested that a secretion of the posterior pituitary gland in the human has an inhibitory effect upon the tubular reabsorption of chloride, and that it is specifically concerned in the control of the excretion of inorganic ions in a manner which is antagonistic to the effect of the secretions of the adrenal cortex.

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CONCENTRATION AND TRANSPORT OF TRUE URATE IN THE PLASMA OF THE AZOTEMIC CHICKEN

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Received for publication August 15, 1947

In birds uric acid is the chief end product of protein metabolism, occupying a position in intermediate metabolism, functionally analogous to that of urea in mammals (1). Renal insufficiency in the duck is associated with the development of extremely high blood urate values (2) rather than the elevated urea values seen in mammals. The plasma urate values found in azotemic birds are many times the accepted maximum true solubility of urate in plasma water (3). The experiments reported in this communication were designed to explore the mechanism by which urate is transported without precipitation in the plasma of the azotemic chicken.

METHODS. Azotemia was induced in young chickens by extraperitoneal bilateral ureteral ligation through paracloacal incisions. Our animals uniformly survived for from 12 to 24 hours as did those studied by Folin, Berglund and Derick (2). After at least 12 hours, when the birds clinically appeared moribund, blood samples were withdrawn in heparinized syringes and either spun immediately for plasma or kept at room temperature for not more than 6 hours before separation. Biochemical methods employed in further treatment of these samples have been described in other publications dealing with urate metabolism (4, 5).

RESULTS. *True urate in normal and azotemic chickens* (*table 1*). In 4 normal chickens the average plasma total urate concentration, as determined by the colorimetric arsenophosphotungstate method, was 5.8 mgm.%. True urate, determined by the uricase method, represented on the average 86% of these values.

The average plasma total urate in 8 azotemic chickens was 304 mgm.% of which slightly more than 93% was true urate. The concentrations of urate in the blood of azotemic birds studied by Folin, Berglund and Derick (2) and those found by the colorimetric procedure in this study appear chiefly to represent increases in true urate.

Ultrafiltrability of urate (*table 1*). The plasma of normal and azotemic chickens was ultrafiltered through cellophane in order to differentiate the diffusible fraction of the plasma urate. In normal chickens, 71% of the plasma true urate was ultrafiltrable through cellophane, while 82% was ultrafiltrable in the azotemic chickens. The average ultrafiltration data for total urate are similar to those for true urate. In the normal plasma an average of 71% of the total urate was ultrafiltrable, while 82% was ultrafiltrable from azotemic chick plasma.

[1] These departments are in part supported by the Michael Reese Research Foundation.

Effect of refrigeration: Tyndall phenomenon. When plasma or plasma ultrafiltrates from azotemic birds are stored overnight in the refrigerator at 0°C., a heavy, white, flocculent precipitate forms. Neither the plasma nor the ultrafiltrate precipitate will completely redissolve on warming to room temperature, but the plasma precipitate will generally redissolve on warming to about 45° to 60°C. The ultrafiltrate precipitate will occasionally redissolve during such

TABLE 1. *Urate partition and ultrafiltrability of true urate in plasma samples from normal and azotemic chickens*

	PLASMA TOTAL URATE	PLASMA CHROMOGEN	PLASMA TRUE URATE	ULTRAFILTRATE TRUE URATE	% TRUE URATE ULTRAFILTRABLE[1]
		Normal chickens			
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
CA	5.9	0.9	5.0	3.9	69
CB	7.0	1.0	6.0	4.7	70
CC	4.6	0.8	3.8	2.9	68
CD	5.7	0.6	5.1	4.2	73
Average	5.8	0.8	5.0	3.9	71
		Azotemic chickens			
C3	241	13	228	190	74
C4	384	18	366	347	85
C5	230	11	219	239	97
C6	293	9	284	247	78
C8	390	28	362	335	83
C9	353	18	335	255	68
C10	260	33	227	237	93
C11	268	18	250	220	88
Average	304	20	284	259	82

[1] In the table above, and elsewhere in the text, *per cent ultrafiltrable* (F) has been calculated as follows: $\frac{\text{Urate}_{UL}}{\text{Urate}_{P}} \times (0.93) \times (0.96) = F$, where Urate_P is the plasma urate concentration in mgm. per 100 cc.; Urate_{UL} is the ultrafiltrate urate concentration in mgm. per 100 cc.; (0.93) is a correction factor dependent upon the difference in water content of plasma and ultrafiltrate; (0.96) is a correction factor for the Donnan effect.

treatment, but more often it is necessary to add one or two volumes of 0.05 N sodium hydroxide to accomplish complete solution.

The volume of ultrafiltrate obtained in our experiments was generally too small to permit analysis of the supernatant from refrigerated samples; but observations were made on the supernatants from 3 refrigerated plasma samples (table 2). The average true urate value of the original plasma was 271 mgm. %, while the supernatants obtained after refrigeration contained an average of 154

mgm. % of true urate. In a single observation upon an ultrafiltrate with an original total urate concentration of 185 mgm. %, the refrigerated supernatant was found to contain 104 mgm. % or 56% of the original concentration. This is similar to the 57% of total urate which remains in the supernatant from refrigerated plasma samples (table 2).

The Tyndall phenomenon was studied by both direct observation and in a darkfield preparation using fresh, simultaneously prepared, normal and azotemic chick ultrafiltrate for comparison. While the normal chick ultrafiltrate showed a few bright points on direct observation, many more were present in the azotemic ultrafiltrate. In addition, the azotemic ultrafiltrate showed a large number of smaller bright points and a brilliantly illuminated hazy background absent in the normal ultrafiltrate. The medium power darkfield observation confirmed

TABLE 2. *Urate partition in plasma samples from azotemic chickens and in the supernatants from refrigerated plasma samples (Refrigerated samples were stored overnight at 0°C.)*

	TOTAL URATE	CHROMOGEN	TRUE URATE
	Plasma samples		
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*
C3	241	13	228
C4	384	18	366
C5	230	11	219
Average	285	14	271
	Supernatants from refrigerated plasma samples		
C3	197	7	190
C4	113	11	102
C5	178	8	170
Average	163	9	154

those made by direct vision; some resolution of the hazy background of the azotemic ultrafiltrate was possible, and the particles were revealed to exhibit definite Brownian movement in some cases. The possible significance of these observations is discussed below.

Uric acid-like chromogen. In the dog and in man, the uric acid-like chromogen has been shown to consist chiefly of chemically uncharacterized purine metabolites of endogenous origin, which behave physiologically much like true uric acid itself (5). Limited data suggest that this may also be true of the chromogen in the chicken. In the plasma of the chicken (table 1) chromogen forms about the same proportion of total urate as in human plasma (5). The striking increase in chromogen during azotemia in the chicken demonstrates its endogenous origin; moreover, the magnitudes of increase of true urate and of chromogen are roughly proportional. Additional facts which suggest the chemical simi-

larity of chromogen and true urate in the chicken are the observation that both are partially precipitated from plasma at 0°C. (table 2) and that both are partially protein-bound. Both chromogen and true urate show, in addition, an increase in percentage ultrafiltrability with increasing plasma concentration.

DISCUSSION. The data presented above confirm earlier findings (2) of markedly elevated blood urate values in azotemic birds and indicate that these values are predominantly due to an increase in true urate. Furthermore, the uric acid-like chromogen in the chicken appears to be of endogenous origin, as in the dog and in man (5).

In both the normal and azotemic chicken most of the plasma urate is ultrafiltrable through cellophane membranes. The concentrations of true urate found in the normal chick ultrafiltrates lie within the accepted limits of the true solubility of urate in plasma water (3, 6, 8). However, similar protein-free ultrafiltrates from the plasma of azotemic chickens contain an average of 259 mgm. % of true urate. As the maximum value proposed for the true solubility of urate in plasma water is 23 mgm. % (6), it is apparent that less than 10% of the true urate in the azotemic ultrafiltrates can be in true solution.

The larger proportion of the true urate in ultrafiltrates from azotemic chick plasma may well be in the colloidal state. At physiological pH, urate forms colloidal solutions whose physico-chemical properties are compatible with those of such ultrafiltrates (3, 8). The behavior of the azotemic chick ultrafiltrate upon cooling and rewarming suggests flocculation of a colloidal urate sol rather than true precipitation. The demonstration of Tyndall effect and Brownian movement further support this interpretation.

The ease with which true urate passes the ultrafilter membrane suggests that, although it may be in the colloidal state when the ultrafiltrate is examined, it actually may have circulated in the plasma of the azotemic chick as a smaller aggregate or urate polymer. The physico-chemical basis for such an opinion is found in Schade's demonstration that a continuum exists between urate solutions chiefly composed of polymeric aggregates and those satisfying rigid criteria for the colloidal state (8). While urate solutions tend to pass from polymeric to colloidal states with ageing, suitable conditions may inhibit or reverse this transformation. Variable polymerization may, in addition, play a physiological role in the regulation of urate metabolism as in the case of higher nucleoprotein derivatives (9). One may quite reasonably suspect that the urate polyserositis seen terminally both in our chickens (10) and in alloxanized pigeons (11) is the result of local tissue factors which disturb the polymeric stability of urate in the impinging extracellular fluid.

An additional portion of the plasma true urate is non-ultrafiltrable in both the normal and the azotemic chicken. In the normals it forms 29% of the plasma true urate, but in the azotemic chicks it represents only 18% of the plasma true urate. This finding would be expected if the non-ultrafiltrable fraction were to represent an adsorption compound between a constant amount of plasma protein and a variable quantity of urate (12, 13). If the non-ultrafiltrable fraction were merely a larger micellar species of urate than the ultrafiltrable polymeric

urate, the non-ultrafiltrable fraction should represent a larger proportion at higher plasma urate concentrations than at lower concentrations (8).

It appears at present, therefore, that true urate may be transported in the plasma of the azotemic chicken in at least three forms. Protein-bound urate constitutes an average of 18% of the plasma true urate. Not more than an additional 10% is transported in true solution. The remainder, more than 70%, is carried as ultrafiltrable polymeric urate. A review of the available renal data (14, 15) appears to indicate that the avian glomerulus must be assumed to be freely permeable to the ultrafiltrable polymeric urate as well as to urate in true solution, although the protein-bound urate is not necessarily ultrafiltrable through the glomerulus of the bird.

Although these studies indicate the presence of a three-phase system for urate transport in the azotemic chicken, they do not establish the existence of an ultrafiltrable polymeric phase at the urate levels which obtain in the plasma of the normal chicken or in man. Physicochemical methods alone do not permit the demonstration of such a system at any urate value below the poorly defined upper limit of true solubility of urate in plasma water.

However, analagous conditions may obtain even at urate concentrations below the upper limit assumed for urate true solubility. Data obtained in man demonstrate that normally only 6% of the plasma true urate passes through the C.S.F. plasma barrier (4). Renal studies in man indicate that only a similarly small fraction of the plasma true urate passes through the human glomerulus (16, 17).

SUMMARY

Earlier reports of greatly elevated blood urate levels in the azotemic bird have been confirmed for the chicken and have been shown chiefly to represent an increase in plasma true urate concentration when studied by the specific uricase method.

The plasma uric acid-like chromogen is also increased in the azotemic chicken, indicating its endogenous origin. Other data show that, as in the dog and in man, the physiological behavior of chromogen in the chicken closely parallels that of true urate.

A consideration of data obtained from the study of plasma and plasma ultrafiltrates by various methods in both the normal and azotemic chicken has suggested that urate may be transported as a three-phase system in the azotemic birds. A small fraction appears to be in true solution while a somewhat larger fraction is transported as protein-bound urate. However, the largest amount, averaging over 70%, is carried as ultrafiltrable polymeric or ultrafiltrable colloidal urate. Physicochemical methods at present, however, do not permit the demonstration of such a three-phase system when the plasma urate value is below the theoretical maximal solubility of urate in plasma water, a poorly defined value.

A review of earlier data on the renal mechanism for urate excretion in the chicken indicates that the renal glomerulus is probably freely permeable to urate in true solution and to ultrafiltrable polymeric urate in this species, but that

protein-bound urate may not be ultrafiltrable through the glomerulus of the chicken.

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CLEARANCE OF ALLANTOIN IN THE RAT AND DOG AS A MEASURE OF GLOMERULAR FILTRATION RATES[1]

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Received for publication August 25, 1947

Allantoin has been found to be the principal urinary end product of purine metabolism in all subprimate mammals (1–5) with the possible exception of the Dalmatian coach hound (4). However, studies concerning the renal clearance of allantoin have not been published, possibly because of earlier difficulties in ascertaining the allantoin content of blood. Accordingly, no information exists concerning the manner in which allantoin is excreted by the kidney.

It therefore seemed worthwhile to determine the renal clearance of this substance in both the rat and the dog. The experimental evidence obtained from these determinations indicated that blood allantoin was excreted solely by the renal glomeruli of these two species without subsequent tubular reabsorption. In other words, the clearance of allantoin, like that of creatinine, appeared to serve as an accurate measure of the rate of glomerular filtration in the rat and dog, despite variations in *a*) the concentration of plasma allantoin or in *b*) the rate of urine flow.

METHODS. Thirty-seven male albino rats (weight range: 275–350 grams) were used in the clearance studies. Twenty-one allantoin clearances were done on 20 of these rats. Twenty-one creatinine clearances were determined on the remaining 17 rats. In the ascertainment of both types of clearance the same procedure as described previously (6) was employed in which blood samples were obtained, before and at the end of a two-hour urine collection.

Five normal male dogs also were used for this study. The allantoin and creatinine clearances of these animals were determined concomitantly. Nine allantoin and creatinine clearances were determined on 4 of these dogs without parenteral administration of allantoin. These allantoin clearances will be described as endogenous allantoin clearances. Eleven allantoin and 9 creatinine clearances were ascertained on 5 dogs whose plasma allantoin had been elevated by the intravenous administration of allantoin. These clearances will be described as exogenous allantoin clearances.

All dogs were anesthetized with pentobarbital sodium, then catheterized and a solution of 5% glucose was given by vein at a rate of 4 cc. per minute until a urine flow of at least 1 cc. per minute occurred. The animals then were given a priming solution composed of 200 mgm. of creatinine in 50 cc. of 5% glucose solution. The latter solution was given at a rate of 3 cc. per minute. Approximately 30 minutes after the sustaining solution had been started, the bladder was washed out, the urine collection begun and the first blood sample taken. The

[1]Aided by a grant from The Public Health Service.

duration of the collection varied from 10 to 30 minutes, at the end of which time a second blood sample was obtained. Both blood and urine samples were analyzed for allantoin and creatinine. The exogenous allantoin clearances were obtained in the same manner, except that the priming and sustaining solutions also contained 200 mgm. % of allantoin.

Creatinine in plasma and urine was determined by the method of Folin and Wu (7). Allantoin was determined according to the technique of Christman, Foster and Esterer (8) on tungstic acid filtrates of plasma and on suitably diluted urine samples. An ice-salt bath at −8°C. to −10°C. was used in the allantoin assay instead of 0°C. suggested by Christman *et al.* A yeast blank and a standard solution were assayed concurrently with all samples. The standards and blanks were manipulated in the same fashion as the samples; their values as read on a Klett-Summerson photoelectric colorimeter (9) varied only slightly from day to day.

RESULTS. A. *The renal clearance of endogenous allantoin in the rat.* The renal clearance of endogenous allantoin was measured 21 times on 20 rats. The mean clearance (see table 1) was found to be 33.7 cc. per hour per 100 grams of body weight (range: 25.8 to 41.7 cc.). The mean plasma allantoin concentration was found to be 1.76 mgm. % (range: 1.20 to 2.25 mgm. %) during the clearance determinations. The mean urine flow was 1.35 cc. per hour per 100 grams of body weight (range: 1.08 to 1.68 cc.)

The creatinine clearance was studied 21 times on 17 rats. The mean clearance (see table 1) was found to be 34.6 cc. per hour per 100 grams of body weight (range: 27.0 to 43.0 cc.). The mean plasma creatinine concentration was 10.50 mgm. % (range: 7.40 to 14.75 mgm. %) during the clearance determinations. The mean urine flow was 1.27 cc. per hour per 100 grams of body weight (range: 1.00 to 1.50 cc.). As pointed out in a previous study (6) the rat's renal hemodynamics vary according to the rate of urine flow. Therefore, in comparing the clearances of allantoin and creatinine, only clearances obtained during similar urine flows may be validly compared.

The above clearance studies indicated that the average renal clearance of endogenous allantoin was of the same magnitude as that of creatinine. Since the latter was found to be a measure of this animal's glomerular filtration rate (6), it appears that the renal clearance of endogenous allantoin also might be a measure of the same renal function in this animal.

B. *The renal clearance of endogenous allantoin in the dog.* The renal clearance of endogenous allantoin was obtained 9 times on 4 dogs. The mean clearance (see table 2A) was 85.5 cc. per minute per square meter of surface area (range: 75.0 to 92.5 cc.) at a mean urine flow of 3.67 cc. per minute (range: 1.46 to 7.55 cc.). The mean plasma allantoin concentration during the clearance determinations was 0.66 mgm. % (range: 0.40 to 1.03 mgm. %).

Nine control creatinine clearances were obtained also on these dogs at the same time that the allantoin clearances were determined. The mean creatinine clearance was 90.0 cc. per minute (range: 76.0 to 101.0 cc.). The mean plasma concentration of creatinine during the clearance determinations was 6.94 mgm. %

(range: 5.50 to 8.50 mgm. %). This approximate equality of the allantoin and creatinine clearances, irrespective of the rate of urine flow, suggested that the kidneys of both the rat and the dog excreted these two substances in a similar manner.

C. *The renal clearance of exogenous allantoin in the dog.* The renal clearance of allantoin was determined 11 times on 5 dogs after the plasma allantoin concentration had been increased above the normal endogenous level of allantoin by the intravenous administration of the substance. Concomitant determinations of the creatinine clearance also were done in most experiments.

TABLE 1. *Allantoin and creatinine clearances in rats*

RAT	ALLANTOIN CLEARANCE			RAT	CREATININE CLEARANC		
	Plasma allantoin	Urine flow*	Allantoin clearance†		Plasma creatinine	Urine flow*	Creatinine clearance†
	mgm. %				*mgm. %*		
70	2.15	1.60	29.2	41B	13.50	1.10	35.5
20	2.25	1.56	31.6	68B	11.20	1.05	30.2
98	2.20	1.10	39.4	32B	8.60	1.00	33.4
78	1.50	1.68	34.7	68B	12.10	1.04	27.0
42	1.90	1.26	32.0	56B	12.45	1.23	30.5
97	2.00	1.24	26.5	69B	14.75	1.50	33.0
05	1.35	1.35	35.6	57B	13.25	1.31	29.4
42	1.80	1.14	36.4	72B	12.00	1.36	37.7
29	1.65	1.08	29.6	43B	10.10	1.32	43.0
32	2.00	1.30	38.9	22B	14.65	1.41	31.0
96	1.35	1.38	41.7	71B	8.40	1.23	40.0
24	1.80	1.33	33.3	18B	10.12	1.39	34.0
39	1.20	1.31	41.3	87B	10.00	1.28	32.1
01	1.75	1.42	41.0	24B	10.60	1.34	41.5
27	1.65	1.25	25.8	89B	7.40	1.44	37.9
52	1.45	1.50	39.2	24B	8.05	1.14	29.2
62	1.55	1.18	27.9	18B	8.50	1.41	39.3
02	1.95	1.50	31.2	87B	7.80	1.45	39.7
49	1.85	1.39	34.5	52B	9.80	1.36	31.8
14	1.80	1.35	28.6	15B	9.75	1.09	38.2
07	1.90	1.50	29.0	83B	7.60	1.26	33.4
Mean	1.76	1.35	33.7		10.50	1.27	34.6
S.D. of mean	±0.36	±0.16	±4.99		±2.25	±0.15	±4.47

* Urine flow expressed in cc. per hour per 100 grams of body weight.
† Clearance expressed in cc. per hour per 100 grams of body weight.

As table 2B indicates, the renal clearance of exogenous allantoin was independent of the plasma allantoin concentration. Thus, although plasma levels varying from 4.16 to 30.00 mgm. % were attained, no essential change occurred in the clearance of allantoin. Furthermore, the average allantoin clearance of this series (89.9 cc. per minute) at an average plasma allantoin concentration of 19.15

mgm. % was not only essentially the same as the average creatinine clearance (89.3 cc. per minute) but also approximately the same as the clearance of endogenous allantoin (cf. table 2A and 2B). In short, the clearance of allantoin in the dog was not only similar to that of creatinine, but it also was independent of

TABLE 2. *Comparison of the renal clearances of endogenous allantoin, exogenous allantoin and creatinine in dogs*

DOG	URINE FLOW	PLASMA ALLANTOIN	ALLANTOIN CLEARANCE*	PLASMA CREATININE	CREATININE CLEARANCE*
A. The clearance of endogenous allantoin and creatinine					
	cc/M.	*mgm. %*		*mgm. %*	
1	2.43	0.71	91.8	8.50	82.5
1	1.46	0.40	88.0	5.50	91.5
2	2.70	0.51	82.5	6.40	91.8
2	2.76	0.45	89.0	7.80	76.0
3	3.24	0.90	75.0	7.30	82.5
3	4.80	1.03	92.5	8.10	95.5
4	4.10	0.75	83.5	5.90	101.0
4	4.00	0.76	76.0	5.80	99.2
2	7.55	0.45	91.5	7.20	90.0
Mean	3.67	0.66	85.5	6.94	90.0
S.D. of mean	±1.67	±0.17	±6.30	±1.03	±6.44
B. The clearance of exogenous allantoin and creatinine					
1	4.68	4.16	89.5	8.95	80.5
2	4.80	5.60	92.0	—	—
3	4.80	6.75	92.0	10.50	94.2
3	5.00	6.80	95.0	10.55	90.0
1	5.70	21.00	89.5	8.50	78.0
2	4.89	24.00	86.0	6.40	95.0
5	6.85	25.40	74.0	7.50	90.0
5	6.40	27.90	79.0	7.40	84.0
4	3.50	29.00	90.0	—	—
1	5.40	30.00	109.0	6.90	95.0
4	5.80	30.00	93.0	6.80	97.0
Mean	5.26	19.15	89.9	8.16	89.3
S.D. of mean	±2.79		±8.5		±6.5

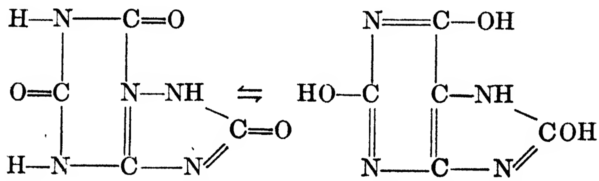
* Clearance expressed in cc. per minute per square meter of surface area.

changes in the plasma concentration of allantoin and in the rate of urine flow. These observations indicated that the allantoin clearance was at the level of glomerular filtration in the dog.

DISCUSSION. The ability of the rat and dog to excrete endogenous allantoin as rapidly as creatinine suggested that the former might be a substance which,

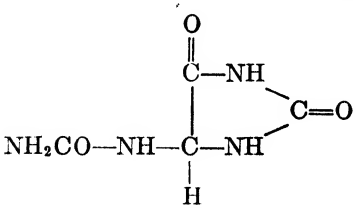
after glomerular filtration, did not undergo subsequent tubular reabsorption. The inability to effect a change in the renal clearance of allantoin in dogs after an elevation of their plasma allantoin content to a level 45 times their average endogenous level indicated without much doubt that the excretion of allantoin was independent of tubular activity. On the basis of these observations then, it seems reasonable to believe that allantoin is excreted solely through the renal glomeruli of the rat and dog without subsequent tubular reabsorption. Consequently, a measurement of its clearance affords one the opportunity of measuring the rate of glomerular filtration in these two species.

The physiological similarity of allantoin to creatinine and its dissimilarity to uric acid (10) in its renal excretion is not surprising when the structural formulae of the 3 substances are observed and compared.

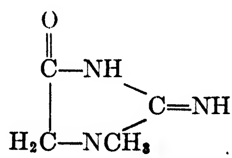


(Keto form) (Enol form)

Uric acid



Allantoin



Creatinine

Despite the fact that allantoin is derived from uric acid, it can be seen from the above formulae that it bears a much closer relationship in a physico-chemical sense to creatinine. For instance, the carbonyl groups at positions 2 and 6 of uric acid are potentially acid because of hydrogen tautomerism to the enol form, while the straight chain ureido carbonyl of allantoin no longer has this acid potentiality, and creatinine is slightly basic. More important perhaps in respect to the differences in renal excretion of these substances, the imidazolidine ring of allantoin and creatinine may be considered as retaining characteristic resonance properties while the imidazole ring of uric acid is fused with pyrimidine, thus having a different electron configuration and different tautomeric possibilities.

SUMMARY

(1) The renal clearance of endogenous allantoin was measured in the rat and found equal to the creatinine clearance.

(2) The renal clearance of endogenous and exogenous allantoin was measured

in the dog. The clearance of allantoin was found to be independent of its concentration in the blood plasma and equal to the clearance of creatinine in the dog.

(3) The clearance of allantoin appears to be at the level of glomerular filtration in the rat and dog.

The authors wish to express their thanks to Barbara Trousdale and Beatrice Bedell, who assisted in these experiments.

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EFFECT OF URETERAL LIGATION, PHLORIDZIN AND MERCURY BICHLORIDE ON THE GLUCOGENIC FUNCTION OF THE KIDNEY[1]

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Received for publication July 7, 1947

Bergman and Drury (1) studied the rate at which it was necessary to inject glucose in order to maintain the blood sugar of an eviscerated rabbit at a normal level. They found that ligating the ureters of this preparation necessitated an increase in the rate of glucose administration. Later, Reinecke (2) found that the kidneys were a source of blood sugar in the eviscerated rat. It follows then that Bergman and Drury's findings may have been due to variations in the amount of blood sugar furnished by the kidneys of their preparation and that ureteral ligation reduced the amount of sugar so furnished. A consideration of the morphology of the kidney suggests that this effect of ureteral ligation may be due to its interference with glomerular filtration and, consequently, that renal glucogenesis is possible only if there is an adequate rate of glomerular filtration.

Phloridzin has long been known to be a rather specific poison for the mechanism by which glucose is resorbed from the glomerular filtrate (3). It is to be expected then that the mechanism by which new sugar is formed in the kidney will also be poisoned by this substance if it is related to that involved in the resorption of sugar.

Mercury bichloride is a general protoplasmic poison that under some circumstances is almost specific for parts of the renal tubule (4). It therefore is likely to stop any process that demands the expenditure of energy by the mechanisms located within these cells. Since the concentration of mercury in the tubule, and hence its specificity of action, may be dependent upon glomerular filtration, it might also be expected that ureteral ligation would decrease the effectiveness of the mercury.

These hypotheses were tested in the non-nephrectomized, eviscerated rat.

The methods employed, except as noted, were those used previously (2). Fermentations were not done; and, therefore, the blood sugar values shown include indeterminate amounts of nonfermentable reducing substances. Blood samples of about 0.15 ml. were drawn simultaneously, where so indicated, from the aorta and left renal vein. Tuberculin syringes (0.25 cc.) fitted with no. 27 needles were used. The syringes were coated internally with light mineral oil. Immediately after withdrawal these samples were discharged into small depressions in paraffin blocks. Quantitatively accurate samples were then conveniently measured for analysis. It was not necessary to use anticoagulants.

[1] Supported by a grant from the John and Mary R. Markle Foundation.

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Some samples, where so identified, were also taken from the heart. All other samples were measured directly from drops of blood obtained from the vessels opened by cutting off a very small bit of the tip of the tail. In the authors' hands, this maneuver is more successful if a sharp scalpel is used to cut off the bit of tissue while the tail is lying on a large cork. Hemoglobin determinations were made on the samples from the renal vein and aorta as a check on the sampling technic. Two hundredths milliliters of blood was measured into 5 ml. of distilled water. A drop of concentrated ammonium hydroxide was added just

TABLE 1[1]

TREATMENT	ANIMAL	BLOOD SUGAR RENAL V.	BLOOD SUGAR AORTA	HEMOGLOBIN OPTICAL DENSITY RENAL V.	HEMOGLOBIN OPTICAL DENSITY AORTA	BLOOD SUGAR RENAL V. / AORTA	HEMOGLOBIN RENAL V. / AORTA
		mgm/100 cc.					
Ureters not ligated	A	47	26	.600	.610	1.81	.98
Ureters ligated	B	19	20	.595	.600	.95	.99
	C	53	36	.620	.610	1.47	1.02
	D	56	37	.585	.585	1.51	1.00
	E	78	63	.585	.595	1.24	.98
Phloridzin; ureters not ligated	F	59	35	.536	.531	1.69	1.01
Phloridzin; ureters ligated	G	70	51	.531	.526	1.37	1.01
	H	80	56	.508	.503	1.43	1.01
	I	89	70	.547	.550	1.27	.99
	J	89	76	.543	.546	1.17	.99
Mercury Bichloride; ureters not ligated	K	32	20	.580	.572	1.60	1.01
	L	35	20	.640	.620	1.76	1.03
	M	30	33	.655	.650	.91	1.01

[1] See figures 2–4 for times after ligation of the arteries at which the samples were taken. The letters designating animals correspond to those given on these figures.

before estimating the optical density in a photoelectric colorimeter with a spectral band, having its maximum at 540 mu wavelength (5).

The first hypothesis developed in the introduction was tested by studying the effect of ureteral ligation on the blood sugar levels of the eviscerated rat. (See fig. 1 and 2 and *animals A–E* in table 1.) In contrast to the finding in the eviscerated rabbit, this maneuver seemed without major effect. The number of instances was too small to make apparent any moderate changes, but qualitatively there was no evidence of decreased renal glucogenesis. If anything, the animals with ligated ureters survived in better condition than their controls. In an experiment in which the blood sugar level in the renal vein was compared with that in the aorta, 3 of the 4 animals with ligated ureters exhibited a definitely higher level in the renal vein. The samples were taken from the fourth animal as it was about to expire.

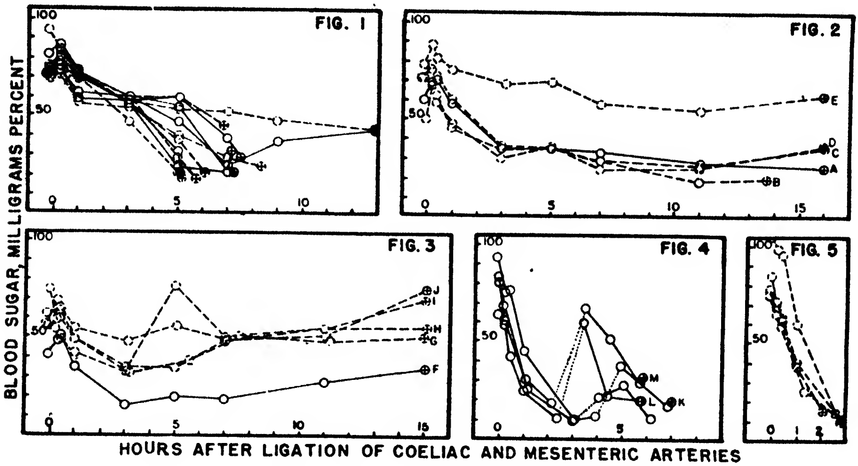

FIG. 1. *Effect of ureteral ligation.* The animals were fasted for 2 days prior to evisceration. Broken lines indicate animals whose ureters were ligated at the time of evisceration. Samples marked with crosses were taken from the heart immediately after death. The other samples were taken from the tail.

FIG. 2. *Effect of ureteral ligation.* The animals were fasted for 4 days prior to evisceration. Broken lines indicate animals whose ureters were ligated at the time of evisceration. Samples marked with crosses were taken from the aorta with the animal under amytal anesthesia. The letters identify the animals listed in table 1, where the samples taken simultaneously from the renal vein are compared with these from the aorta. The other samples were taken fron the tail.

FIG. 3. *The effect of phloridzinization.* The animals were fasted for 5 days prior to evisceration. They were given doses of 50 mgm. of phloridzin suspended in peanut oil subcutaneously 4 days before evisceration and again on the day before evisceration. Urine samples, collected from the renal pelves at the end of the experiment from the animals whose ureters had been ligated, all gave a positive test with Benedict's qualitative reagent. Urine was voided twice after evisceration by the animal whose ureters had not been ligated. Both samples gave a positive test for sugar with the reagent mentioned. The significance of the broken lines, letters and crosses is as given for figure 2.

FIG. 4. *Effect of mercury bichloride.* The animals were fasted for 4 days prior to evisceration. They were given 20 mgm./kgm. of mercury bichloride in 1/1000 solution in saline subcutaneously 2–3 hours before evisceration. After an animal had exhibited a convulsion and was about to expire, it was given intravenously 0.25 ml. of a 25-gram % glucose solution per 100 gram of body weight (eviscerated). The animals regained consciousness and were able to sit up within a few minutes after this treatment. The blood sugar curves for before and after this treatment are connected by dotted lines. The significance of the letters and crosses is as given for figure 2.

FIG. 5. *The effect of mercury bichloride after ureteral ligation.* The animals were fasted for 4 days prior to evisceration. Their ureters were ligated under ether anesthesia about 4 hours before evisceration, and they were given 20 mgm./kgm. of mercury bichloride in saline subcutaneously about two hours before evisceration. The samples marked with crosses were taken from the heart immediately after death. All of these animals exhibited convulsions before death.

Phloridzinization likewise was found to be without any marked effect on sugar formation in the kidney. (See fig. 3 and *animals F–J* in table 1.) The animals survived well. They maintained blood sugar levels that seemed higher than we usually find in the eviscerated rat and exhibited a definite increase in the blood sugar concentration in the renal vein as compared with that in the aorta. One of the animals given phloridzin exhibited all of these findings, save that its sugar levels were lower, in spite of the fact that its ureters had not been ligated and it was demonstrably losing quantities of sugar in its urine.

Mercury bichloride administration promptly caused the eviscerated animals to develop a fatal hypoglycemia. (See fig. 4 and 5 and *animals K–M* in table 1.) Ureteral ligation did not interfere with this action of mercury. Somewhat surprisingly, however, the mercury did not prevent the appearance of a higher blood sugar level in the renal vein as compared with the aorta in 2 of the 3 animals studied.

The lack of effect of ureteral ligation in preventing either renal glucogenesis or mercury poisoning may be interpreted alternatively as indicating either that ureteral ligation does not significantly reduce the rate of glomerular filtration or that this process is not necessary for these phenomena to occur. Since a search of the literature failed to yield any pertinent evidence on this point, further experimental work will be needed to resolve the issue. The result is, however, of some practical significance, for now it is clear that ureteral ligation can be used to eliminate the loss of substances in the urine when the renal glucogenic function is under study in this species. This consideration, for instance, was useful in the study of the effect of phloridzin. The lack of correlation of this finding in the rat with that of Bergman and Drury in the rabbit suggests a species difference, but it may merely be due to a difference in the experimental approach.

The simplest explanation of the results of the studies on the effects of phloridzin would seem to be that the phenomenon of renal glucogenesis is not intimately related to that involved in the resorption of glucose from the glomerular filtrate.

It is evident that the action of mercury bichloride is to make the sources of blood sugar in the eviscerated, non-nephrectomized rat inadequate to meet its metabolic needs. Since the renal blood flow was not measured, it is not apparent whether this was due to increased utilization or decreased production. Under the conditions of this experiment it is clear, however, that mercuric chloride does not entirely stop renal glucogenesis.

SUMMARY

Ureteral ligation and phloridzinization did not prevent the kidney from acting as a source of blood sugar in the eviscerated rat. Administration of mercury bichloride led to the prompt development of hypoglycemia in this preparation.

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RENAL CLEARANCE OF ESSENTIAL AMINO ACIDS: THEIR COMPETITION FOR REABSORPTION BY THE RENAL TUBULES

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Received for publication August 2, 1947

The essential amino acids fall broadly into two categories with respect to their renal tubular reabsorption when administered singly to unanesthetized dogs: *a*) those whose reabsorption is essentially complete at all technically feasible plasma levels, and *b*) those that can be demonstrated to have a fairly well-defined maximal reabsorptive rate or *Tm*, as was first shown to be the case for arginine by Pitts (1). The following amino acids may be classed in group 1: tryptophane, leucine, isoleucine, valine, histidine, methionine, threonine and phenylalanine (2, 3, 4). The amino acids arginine and lysine are in group 2 (3).

Knowledge gained in other studies of renal physiology in which substrates have been reported to compete for a common functional transport mechanism has suggested the possibility that a similar competition may exist among amino acids for reabsorption by one or more processes. If such a state of competition or interference exists, then the reabsorption of individual amino acids administered as mixtures or as protein hydrolysates may be quite different quantitatively from what might be anticipated on the basis of the above classification and literature.

It has been the purpose of this study to administer certain pairs of amino acids together and to evaluate their competition for reabsorption by the tubules. Such a competition will be reported herein to hold for certain amino acids and not for others. As in our previous work, we have made use of microbiologic methods for the specific determination of the amino acids studied. A preliminary report of these findings has been made (5).

METHODS. In general the methods used in this study were similar to those reported previously (2–5). The clearances were determined on three unanesthetized dogs trained to lie lightly restrained in a comfortably padded cradle during the experiments. Creatinine was administered subcutaneously in a 3.0 gram dose before the study since its clearance is equivalent to glomerular filtration rate in the dog. Para-aminohippurate was administered orally in a dose of 100 mgm./kgm. one to two hours before beginning the clearance periods. Its clearance is equivalent to minimal renal plasma flow and the oral administration permits a sustained plasma concentration in dogs of about 1 mgm./100 cc. over a period of at least 3 hours. To condense the tables all PAH clearances have been omitted except as a token in table 4, since they did not contribute materially to the interpretation of these data. The amino acids were administered intravenously by an electrically driven infusion apparatus at a definite rate of 3 to 12 cc./min. and in an amount indicated in the tables for the various experiments.

The methods used for the microbiologic assay of the amino acids have been referred to previously (2, 3, 4). Assays on suitably diluted urine samples were carried out without previous treatment. Determinations of the amino acids in plasma were carried out on protein-free filtrates prepared according to the method of Dunn *et al.* (6).

These studies have involved the coadministration of *a*) two amino acids well reabsorbed at high plasma concentrations; *b*) two amino acids relatively poorly reabsorbed at high plasma levels; or *c*) two amino acids one of which was poorly reabsorbed, and the other well reabsorbed, when administered alone.

Results and discussion. *Competition between two individually well-reabsorbed amino acids* has been demonstrated herein for leucine and isoleucine (table 1). The design of this experiment was such that in the initial phase the

Table 1. *Interference of leucine with the tubular reabsorption of isoleucine*

Dog 1, wt. 17.2 kgm.

CREATININE CLEARANCE	URINE FLOW	l(−)-LEUCINE				dl-ISOLEUCINE			
		Plasma conc.	Amount filtered	Amount reabsorbed	Clearance	Plasma conc.	Amount filtered	Amount reabsorbed	Clearance
Leucine dosage: priming, 4.0 mgm/kgm., i.v.; maintenance, 8.0 mgm/kgm/min., i.v. for the duration of the experiment. Infusion rate, 6.0 cc/min. Equilibration period for leucine before initial clearance, 30 min.									
cc/min.	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*
68.5	5.2	0.43	29.46	29.43	0.08	0.026	1.78	1.77	0.26
75.6	5.7	0.50	37.80	37.74	0.11	0.023	1.74	1.73	0.31
Isoleucine dosage: priming, 2.0 mgm/kgm., i.v.; maintenance, 4.0 mgm/kgm/min., i.v. Equilibration period for isoleucine plus leucine before next clearance period, 30 min.									
72.4	11.3	1.05	76.02	64.70	10.78	0.27	19.33	15.26	15.26
70.1	11.9	0.99	69.40	53.28	16.29	0.35	24.74	19.74	14.18

plasma concentration of leucine was elevated to determine its effect on the clearance of endogenous isoleucine over successive 10-minute periods. In the second phase the maintenance infusion of leucine remained constant but the plasma concentration of isoleucine was elevated by the intravenous coadministration of isoleucine with leucine. After a 30-minute equilibration period duplicate 10-minute clearances of both compounds were obtained.

The clearances of the two compounds at the lower plasma levels were similar to those observed previously in that the values were less than 1.0 cc/min. However, in the second phase, at a plasma concentration of approximately 0.3 mgm/cc., the clearance of isoleucine increased to about 15 cc/min., and the reabsorption of the compound decreased to 75 %. This latter finding was in sharp contrast to the results obtained when isoleucine was administered alone to dogs. Previously we found that at plasma concentrations up to 1 mgm/cc., the clearance of isoleucine administered alone was only 1.1 cc/min. (2). It seems clear then that the coadministration of leucine decreased the reabsorptive capacity of the tubules for isoleucine.

Previously published data on leucine have been inadequate for similar comparisons, since the plasma concentrations were lower than those reported herein. In more recent unreported experiments we have found that at plasma concentrations of 1.0 mgm/cc., the clearance of leucine administered alone was about 13 cc/min., or approximately that observed in the present experiments where leucine was administered with isoleucine. The results indicate that in a competition between these amino acids for reabsorption by the renal tubules leucine is reabsorbed at the expense of isoleucine.

Competition between two relatively poorly reabsorbed amino acids has been demonstrated for arginine and lysine. The design of the experiment was fundamentally the same as that of the previous one for leucine and isoleucine and has been incorporated as a protocol in table 2. Essentially we have determined the effect

TABLE 2. *Competition between arginine and lysine for tubular reabsorption*
Dog 2, wt. 13.9 kgm.

CREATININE CLEARANCE	URINE FLOW	l(+)-ARGININE				l(+)-LYSINE			
		Plasma conc.	Amount filtered	Amount reabsorbed	Clearance	Plasma conc.	Amount filtered	Amount reabsorbed	Clearance
Arginine dosage: priming, 4.0 mgm/kgm., i.v.; maintenance, 7.0 mgm/kgm/min., i.v. for the duration of the experiment. Infusion rate, 3.0 cc/min. Equilibration period of arginine infusion before initial clearance, 25 min.									
cc/min.	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*
67.7	7.7	0.31	20.85	9.67	36.29	0.048	3.25	1.75	31.3
67.0	8.2	0.38	25.73	9.46	42.37	0.051	3.42	1.93	29.2
Lysine dosage: priming, 4.0 mgm/kgm., i.v.; maintenance, 7.0 mgm/kgm/min., i.v. *Arginine* dosage: maintenance, 7.0 mgm/kgm/min., i.v. Equilibration period of arginine plus lysine infusion before next clearance, 25 min.									
58.0	10.4	0.54	31.15	3.05	52.32	0.40	23.03	3.13	50.10
59.7	10.3	0.61	36.60	5.10	51.38	0.46	27.70	2.64	54.00

of arginine, at filtration loads sufficient to measure its *Tm*, on the reabsorption and clearance of endogenous lysine. In addition, the experiment was designed to demonstrate the mutual effect of the two compounds at elevated plasma concentrations on their respective reabsorptive capacities.

In the light of our previous experience with these two compounds studied singly (3), several findings are noteworthy. The reabsorption of arginine at elevated plasma concentration, or filtration load, suppressed the tubular reabsorption of endogenous lysine significantly. This effect is the more impressive if it be recalled that at endogenous plasma concentrations of lysine alone its clearance was 0.14 cc/min. as compared with clearances of about 30 cc/min. (table 2) in the present study at similar plasma concentrations.

The *Tm* for arginine in this animal in this and other trials was about 9 to 11 mgm/min. *Tm* values for lysine have been found to be 10 to 14 mgm/min. in this and other dogs of this size or larger. However, in the second phase of the

experiment, summarized in table 2, the *Tm* values for both compounds were depressed greatly, as represented by the rates of reabsorption at elevated plasma concentration. Whereas the average filtration rate for lysine increased 7.6-fold at the elevated plasma concentration, its reabsorption by the tubules increased only 1.6-fold over the rate of reabsorption of endogenous lysine. The plasma concentration of 0.40 to 0.46 mgm/cc. has been found to be sufficient for the measurement of lysine *Tm* when that compound was administered alone. Similarly, the amount of arginine that was reabsorbed decreased when coadministered with lysine, even though its plasma concentration and filtration rate increased. The decreased total mgm/min. reabsorption of the two compounds in the second phase below that obtaining in the first part of the experiment has not been found consistently. Frequently the sum of the reabsorptions of the two compounds at elevated plasma level approximates the *Tm* for one of the amino acids instead of the sum of their *Tm* values. This may be considered as at least presumptive evidence that arginine and lysine are reabsorbed by a common transport mechanism. The decreased creatinine clearance in the second phase of table 2 is not representative for the three dogs or repeated experiments on the same animal.

Competition between two amino acids, one of which is poorly reabsorbed and the other well reabsorbed when administered alone, has been demonstrated between arginine and histidine (table 3). In this experiment clearances of endogenous arginine and histidine were determined followed by clearances of the two amino acids after plasma levels of both compounds had been elevated in increments by the use of intravenous infusion of the two compounds in the dosages indicated.

At plasma levels of arginine of approximately 0.15 mgm/cc., resulting in the filtration of 12–13 mgm/min. of arginine, 11–12 mgm/min. were reabsorbed. With further elevation in plasma level the simultaneous administration of histidine resulted in a reduction in the capacity of the tubules to reabsorb arginine so that at a plasma level of about 0.40 mgm/cc., resulting in a filtration of about 30 mgm/min. of arginine, only 6 mgm/min. were reabsorbed. It will be recalled that when arginine is administered alone the dog is capable of reabsorbing about 11 mgm/min. (15 mgm/min/sq. meter). Clearly then the coadministration of histidine with arginine resulted in a decreased capacity to reabsorb arginine.

Published data on the clearances of histidine have not been carried out at plasma levels sufficiently high for interpretation of the histidine data of the present experiment. However, subsequent unpublished experiments have shown that the plasma level of histidine in dogs may be raised to 1 mgm/cc., resulting in the filtration of 55 mgm/min. of histidine, with clearance of 1 cc/min. or less. In the present experiment where plasma levels of only 0.50–0.60 mgm/min. were obtained, in the presence of similar plasma levels of arginine, histidine clearances of 13–16 cc/min. were observed. Thus there was a reciprocal decrease in the reabsorptive capacity of the tubules for both arginine and histidine when the two amino acids were administered together.

Instances wherein interference with reabsorption could not be demonstrated when two amino acids were infused together. Table 4 summarizes an experiment wherein the effect of glycine on the reabsorption of isoleucine was studied. We have not

TABLE 3. *Competition between arginine and histidine for tubular reabsorption*
Dog 3, wt. 14.9 kgm.

CREATININE CLEARANCE	URINE FLOW	l(+)-ARGININE				l(+)-HISTIDINE			
		Plasma conc.	Amount filtered	Amount reabsorbed	Clearance	Plasma conc.	Amount filtered	Amount reabsorbed	Clearance
Control: post-absorptive but after priming dose of water									
cc/min.	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*
78.3	1.75	0.018	1.40	1.39	0.54	0.018	1.40	1.39	0.36
59.4	0.64	0.016	0.95	0.94	0.50	0.016	1.06	1.05	0.33
Arginine and *histidine* dosage: priming, 2 mgm/kgm., i.v.; maintenance, 4 mgm/kgm/min., i.v. Infusion rate, 3 cc/min. Equilibration period of infusion before initial clearance, 25 min.									
85.9	4.4	0.147	12.63	12.01	4.21	0.135	11.59	11.50	0.70
74.3	6.8	0.168	12.48	11.04	8.57	0.152	11.29	11.12	1.15
Arginine and *histidine* dosage: priming, 4 mgm/kgm., i.v.; maintenance, 6 mgm/kgm/min., i.v. Infusion rate, 3 cc/min. Equilibration period of infusion before initial clearance, 25 min.									
68.7	8.3	0.264	18.14	9.15	34.05	0.282	19.37	18.43	3.30
61.5	4.9	0.307	18.88	8.81	32.80	0.310	19.06	17.65	4.54
Arginine and *histidine* dosage: priming, 6 mgm/kgm., i.v.; maintenance, 8 mgm/kgm/min., i.v. Infusion rate, 3 cc/min. Equilibration period of infusion before initial clearance, 25 min.									
72.0	1.7	0.376	27.07	6.14	55.66	0.540	38.88	31.82	13.05
73.6	1.7	0.442	32.53	6.72	58.39	0.657	48.35	37.68	16.25

TABLE 4. *Absence of interference of glycine with the tubular reabsorption of isoleucine*
Dog 1, wt. 17.5 kgm.

RENAL PLASMA FLOW	CREATININE CLEARANCE	URINE FLOW	dl-ISOLEUCINE				
			Plasma conc.	Amount filtered	Amount reabsorbed	Amount excreted	Clearance
Glycine dosage: priming, 4.0 mgm/kgm., i.v.; maintenance, 8.0 mgm/kgm/min., i.v. for the duration of the experiment. Rate of infusion, 3.0 cc/min. Equilibration period for glycine infusion before first clearance, 20 min.							
cc/min.	*cc/min.*	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*mgm/min.*	*cc/min.*
207	87.1	4.7	0.068	5.92	5.92	0.003	0.04
220	95.7	5.1	0.058	5.55	5.55	0.004	0.07
Isoleucine dosage: priming, 2.0 mgm/kgm., i.v.; maintenance, 4.0 mgm/kgm/min., i.v. Equilibration period for glycine plus isoleucine infusion before beginning next clearance period, 30 min.							
237	83.8	8.0	0.330	27.65	27.34	0.31	0.95
176	74.3	7.3	0.355	26.38	25.96	0.42	1.19

determined glycine microbiologically; consequently, in this and in subsequent experiments involving glycine administration, interpretation is limited to the effect of glycine on the behavior of the amino acid determined. The effect of glycine on the clearance of isoleucine was determined at endogenous and elevated plasma concentrations of the latter compound. This experiment may be compared with that for leucine and isoleucine represented in table 1; since the same dog was used, the rates of infusion of glycine and leucine were the same, and the plasma levels for isoleucine were essentially the same in the two instances.

TABLE 5. *Absence of competition between arginine and leucine for tubular reabsorption*
Dog 3, wt. 14.0 kgm.

CREATININE CLEARANCE	l(+)-ARGININE				(l−)-LEUCINE			
	Plasma conc.	Amount filtered	Amount reabsorbed	Clearance	Plasma conc.	Amount filtered	Amount reabsorbed	Clearance
Endogenous control clearances								
cc/min.	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*
59.2	0.052	3.08	3.07	0.10	0.0072	0.42	0.42	0.09
62.2	0.064	3.98	3.97	0.12	0.0054	0.34	0.34	0.19
Arginine dosage: priming, 2.0 mgm/kgm., i.v.; maintenance, 2.0 mgm/kgm/min., i.v. for the duration of the experiment. Infusion rate, 6.0 cc/min. Infused for 15 minutes before next clearances were performed								
57.0	0.12	6.95	6.94	0.05	0.068	3.88	3.88	0.01
52.2	0.14	7.31	7.30	0.04	0.073	3.81	3.81	0.02
Leucine dosage: priming, 2.0 mgm/kgm., i.v.; maintenance, 4.0 mgm/kgm/min., i.v. Infusion of arginine plus leucine for 15 minutes before next clearances were performed								
57.0	0.18	10.09	10.08	0.06	0.15	8.78	8.76	0.09
53.7	0.19	10.10	10.09	0.06	0.18	9.45	9.44	0.08
Leucine dosage: priming, 4.0 mgm/kgm., i.v.; maintenance, 8.0 mgm/kgm/min., i.v. Infusion of arginine plus leucine for 15 minutes before next clearances were performed								
50.0	0.19	9.45	9.44	0.06	0.32	16.10	16.07	0.08
62.4	0.17	10.80	10.79	0.08	0.42	26.40	23.36	0.09

It is quite clear from this comparison that glycine exerted no demonstrable effect on the reabsorption of isoleucine at endogenous or elevated plasma concentrations of the latter amino acid. Since the amount of isoleucine reabsorbed per minute in this experiment was materially greater than in the experiment summarized in table 1, the data lend additional support to the thesis that the coadministration of leucine in large amounts depressed the reabsorption of isoleucine. Similarly, it has been demonstrated that glycine did not alter the reabsorption of leucine at rates of infusion that did not produce general systemic effects.

It can be demonstrated that arginine and leucine do not interfere with the reabsorption of each other (table 5). There was no indication of an impairment

of leucine reabsorption by arginine in this or in other experiments in which the plasma concentration and the amount of either or both compounds presented to the tubules for reabsorption was increased several-fold.

Arginine reabsorption has been reported by Pitts to be impaired by glycine (1). We have studied its effect on arginine reabsorption, as illustrated by the experiment summarized in table 6.

TABLE 6. *The effect of glycine on the tubular reabsorption of arginine*
Dog 1, wt. 16.7 kgm.

CREATININE CLEARANCE	URINE FLOW	l(+)-ARGININE			
		Plasma conc.	Amount filtered	Amount reabsorbed	Clearance
Arginine dosage: priming, 2.0 mgm/kgm., i.v.; maintenance, 2.0 mgm/kgm/min., i.v. throughout the experiment. Equilibration period for arginine infusion before first clearance, 15 min.					
cc/min.	*cc/min.*	*mgm/cc.*	*mgm/min.*	*mgm/min.*	*cc/min.*
76.8	5.5	0.10	7.76	7.74	0.17
71.4	5.2	0.12	8.35	8.21	1.23
Glycine dosage: priming, 2.0 mgm/kgm/min., i.v.; maintenance, 4.0 mgm/kgm/min., i.v. Equilibration period for arginine plus glycine infusion before next clearance, 15 min.					
75.1	5.6	0.14	10.51	10.14	2.67
71.2	5.5	0.16	11.18	10.71	3.03
Glycine dosage: priming, 3.0 mgm/kgm/min., i.v.; maintenance, 8.0 mgm/kgm/min., i.v. Equilibration period for arginine plus glycine infusion before next clearance, 15 min.					
70.6	6.0	0.16	11.44	10.11	8.21
79.7	6.1	0.15	11.72	10.74	6.69
Glycine dosage: priming, 4.0 mgm/kgm/min., i.v.; maintenance, 16.0 mgm/kgm/min., i.v. Equilibration period for arginine plus glycine infusion before next clearance, 15 min.					
66.6	6.7	0.13	8.39	7.31	8.57
67.1	7.0	0.12	8.32	7.27	8.47

In this experiment we have attempted to hold the plasma concentration for arginine constant at a level that would just make possible the determination of *Tm*. If glycine inhibited the reabsorption of arginine, the *Tm* value should be depressed out of proportion to any variation in glomerular filtration. The plasma concentration of glycine was increased step-wise by progressive increases in the amount infused per minute. In the last phase of the experiment the infusion of glycine was twice (16 mgm/kgm/min.) any infusion rate for other compounds studied in this series. From such an experiment as represented in table 6, it would be difficult to conclude without reservation that glycine materially interfered with the reabsorption of arginine as determined microbiologically. Certainly such a competition as, for example, that between arginine and lysine, is not demonstrable.

It appears that there may be no single relationship that holds for the interference of amino acids with the reabsorption of each other. Some pairs of compounds such as arginine and lysine, arginine and histidine, or leucine and isoleucine do compete with each other for reabsorption when the amount of the compounds presented to the tubules for reabsorption approaches the functional capacity of the cells to reabsorb one of that pair of amino acids.

The results of the present paper have been summarized diagrammatically in figure 1, in which the amino acids are placed into groups with respect to the presence or absence of demonstrable competition among them for tubular reabsorption. These results suggest, but do not prove, that several selective mechanisms may be involved in the tubular reabsorption. One mechanism appears to be concerned with the basic amino acids, arginine, histidine and lysine. A second transport mechanism seems to be concerned with at least the mono-amino-monocarboxylic amino acids, leucine and isoleucine. A third mechanism

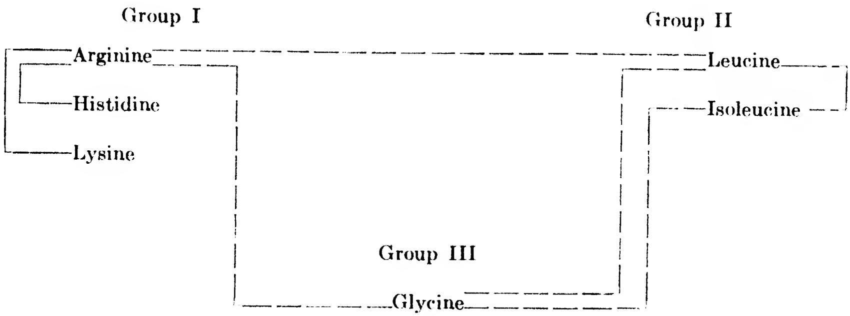

Fig. 1. Classification of amino acids into groups with respect to probable mechanism of reabsorption. —— indicates established interference. - - - - - - indicates no demonstrable interference.

may be concerned with glycine transport, since it was not possible to demonstrate that the administration of this amino acid in large amounts interfered with the tubular reabsorption of amino acids from either of the two established groups.

SUMMARY

Competition for reabsorption by the renal tubules was demonstrated to exist following the intravenous administration of the amino acid pairs arginine and lysine, arginine and histidine, leucine and isoleucine. No competition for reabsorption was observed with the amino acid pairs arginine and glycine, leucine and glycine, isoleucine and glycine, and arginine and leucine. The results are interpreted to indicate the existence of at least two tubular mechanisms for the reabsorption of amino acids.

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ENDOGENOUS AND EXOGENOUS CREATININE CLEARANCES IN THE RAT

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Received for publication August 1, 1947

There have been few determinations of renal clearances in the rat. The rat is a small animal, and because of this it is difficult to collect urine samples with accuracy and to collect blood without producing shock. However, because the rat is small it is useful for investigations involving the use of substances which cannot be obtained in quantity. This study was undertaken as one in a series, investigating the use of clearances as a measure of renal function in the rat. The results presented here indicate that at endogenous levels of serum creatinine concentration, the creatinine clearance is much lower than at high serum concentrations produced by injection of creatinine.

METHODS. In this study we used 191 normal albino rats of both sexes over a wide weight range. Creatinine was determined in serum and urine by a modification of the Folin-Wu method described by Barrett and Addis (1).

Clearances were performed on groups of 5 to 10 rats of uniform sex and body weight. The rats were placed on a diet containing 10% dextrose and 1% of a vitamin B complex in 0.4% salt solution at 4:30 P.M. on the day preceding the experiment. In the morning the rats were lightly anesthetized with ether, the tails were cut, and 0.5 cc. of blood was collected for blank determinations. A rubber tourniquet was used to prevent subsequent blood loss. Then the material for clearance determination was injected subcutaneously into both scapular regions in a volume of 10 cc. Each rat was timed individually, and at the desired time after injection the rats were lightly etherized, causing the bladders to empty. The tourniquets were removed and, after warming the tail, 1.0 to 1.5 cc. of blood were obtained by free bleeding. The tourniquets were replaced and urine was collected for one hour.

At the end of the collection period the rats were again anesthetized, causing the bladders to empty and ending the collections. They were then exsanguinated by severing the abdominal aorta. Kidney weights and urine volumes were measured, and body weights were obtained just before injection of fluid. Chemical determinations were made on pooled specimens of serum and urine. Clearances were calculated on the basis of mid-point serum concentrations obtained from the mean of the log concentration at start and finish of the collection period.

Exogenous creatinine clearances were obtained by injecting 50 mgm. of creatinine in 10 cc. of 0.85% salt solution. The mid-point serum concentration was

[1] The author expresses grateful appreciation for the technical assistance of Evalyn Barrett and William Lew.

varied by changing the interval between time of injection and time of starting collection from ½ hour to 5½ hours. The dose of creatinine remained constant. Endogenous creatinine clearances were obtained by the use of 10 cc. of 0.85% salt solution alone.

TABLE 1. *Endogenous and exogenous creatinine clearances in the rat*

NO. RATS IN GROUP	MEAN G.BW	MEAN MGM.KW	MEAN URINE VOL. CC/G.KW/MIN.	MEAN CREAT. EXCRETION MGM./G.KW/MIN,	MIDPOINT SERUM CREAT. MGM.%	CLEARANCE CC/G.KW/MIN.
			Endogenous			
10	181	1029	0.036	0.00314	0.76	0.413
9	187	1122	0.042	0.00276	0.78	0.354
7	174	1088	0.023	0.00347	0.79	0.439
9	179	1331	0.010	0.00226	0.85	0.265
8	179	1108	0.018	0.00309	0.87	0.354
9	179	1063	0.036	0.00402	0.91	0.441
10	184	1341	0.015	0.00345	1.05	0.328
			Intermediate exogenous			
7	182	1091	0.023	0.00960	1.28	0.750
9	185	1201	0.024	0.00976	1.32	0.730
7	185	1042	0.021	0.0153	1.48	1.03
10	179	1070	0.028	0.0149	1.52	0.980
			Exogenous			
8	360	2059	0.032	0.0386	2.77	1.39
5	170	1150	0.025	0.0532	3.76	1.41
7	174	1217	0.023	0.0455	3.80	1.20
5	190	1321	0.030	0.0663	4.02	1.65
6	171	1131	0.021	0.0697	4.07	1.71
6	184	1279	0.031	0.0564	4.09	1.38
7	177	1195	0.028	0.0608	4.22	1.44
5	180	1102	0.029	0.0570	4.68	1.22
8	181	1236	0.020	0.0657	4.72	1.39
8	186	1047	0.022	0.0589	4.90	1.20
10	186	1178	0.023	0.0663	5.06	1.31
6	181	1162	0.018	0.204	11.8	1.74
7	179	1088	0.020	0.194	13.4	1.45
8	176	1037	0.024	0.204	14.0	1.45

Pooled material from groups of rats was used in order to minimize individual variations. In addition, this permits the use of larger samples for determinations, thus increasing the accuracy of procedures.

Because any large population of rats contains some individuals with gross kidney abnormalities, each animal was autopsied at the kill, prior to pooling material. The specimens from those with gross renal abnormalities, such as advanced cystic kidneys, were excluded from the pool. In addition, those ani-

mals which did not bleed freely from the tail without pressure, those which bled inadvertently during the collection period and those which had extremely low urine volumes during the hour of collection were also excluded.

Insofar as structural factors are concerned, clearance varies with the glomerular filtration surface and with the tubular mass, depending upon the substance cleared. It seems obvious that the most direct reference for correction with respect to these factors is the weight of the kidney. In the rats we used, kidney weight varied not directly as body weight but as the 0.72 power of body weight (2). Therefore, we have expressed clearance in terms of the observed kidney weight (KW). Since most other investigators have expressed their results in terms of body weight (BW), we have given our results in these terms also for purposes of comparison.

RESULTS. The endogenous creatinine clearance had a mean value of 0.371 cc./G.KW/min. obtained at serum levels varying from 0.79 to 1.05 mgm.% (table 1). In terms of body weight, this was equivalent to 0.237 cc./100G.BW/min. In contrast, exogenous creatinine clearances obtained at serum creatinine levels varying from 2.77 to 14.05 mgm.% had a mean value of 1.43 cc./G.KW/min. In terms of body weight this was equivalent to 0.915 cc./100G.BW/min. Exogenous creatinine clearances at intermediate serum levels gave intermediate figures.

Although the range of serum concentrations over which the creatinine clearance changes is small, the change itself is striking, a fourfold increase in passing from endogenous to low exogenous levels.

COMMENT. Friedman (3) has obtained exogenous creatinine, inulin and p-aminohippurate clearances in the rat. He found that all of these clearances varied with urine flow, but that at any given level of flow the inulin and exogenous creatinine clearances were identical. The highest rates of urine flow he obtained were comparable to ours, and at this level he found a creatinine clearance of 0.697 cc./100G.BW/min. and a p-aminohippurate clearance of 3.48 cc./100G.BW/min. We did not study the change in clearance with urine flow but maintained a uniformly high rate of flow with little variation. Friedman's suggestion that it would be more correct to say that the clearances and urine flow all vary with the renal blood flow seems reasonable. But we cannot agree with Friedman's statement that the creatinine clearance is independent of serum concentration, although this appears to be true at levels above 2 mgm.%. Our differences may be a consequence of the facts that Friedman used whole blood for creatinine determinations and did not investigate endogenous clearances.

The difference between endogenous and exogenous clearances might be explained by the presence in rat serum of a non-creatinine chromogen in the Jaffe reaction. If this were so, one would expect the clearance to rise to an asymptotic level as the serum concentration rose and the proportion of non-creatinine chromogen fell. This may be true in part, but to make the endogenous and exogenous creatinine clearances equal it would be necessary to assume that almost all the color formed by normal rat serum in the Jaffe reaction, equivalent to about 0.7 mgm.%, is due to non-creatinine chromogen, and this appears unlikely.

SUMMARY

1. Creatinine clearances in the rat were found to increase about fourfold as the serum concentration of creatinine increased from endogenous levels to about 2.0 mgm.%, while at levels above 2.0 mgm.% they remained relatively constant.

2. At endogenous levels, the mean creatinine clearance was found to be 0.371 cc./G.KW/min. At serum concentrations above 2.0 mgm.%, the mean creatinine clearance was found to be 1.43 cc./G.KW/min.

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EFFECT OF 2,3-DIMERCAPTOPROPANOL ON DIURESIS[1,2]

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Received for publication July 28, 1947

The value of 2,3-dimercaptopropanol (British Anti-Lewisite, or BAL) for counteracting the various effects of mercury in both man and experimental animals has been amply demonstrated (1, 2). It seemed likely that BAL also would interfere with the action of mercurial diuretics. During the course of experiments designed to examine this possibility in dogs, it was found that BAL cannot only prevent or interrupt mercupurin diuresis, but also can inhibit water diuresis. The present report is concerned with the antidiuretic effects of BAL and with a consideration of its mechanism of action.

Methods. Experiments were performed in three well-trained unanesthetized female dogs. Each dog was used for an experiment not more than once weekly and was kept on a constant water and food intake.

All experiments followed the basic plan shown for a control study in table 1. In each instance, the experiment was begun at 9 a.m., 22 hours after the last meal. Twenty ml. of water per kilogram of body weight was given through a stomach tube at zero time. Urine was collected approximately every 20 minutes by catheter with bladder washes. Rates of urine flow, glomerular filtration (creatinine clearance) and excretion of chloride were measured during each experiment. In many experiments renal plasma flow (p-amino hippurate clearance) was also measured. In addition, plasma chlorides and the hematocrit were measured at the beginning of each experiment and at intervals thereafter.

Mercupurin was given by intravenous injection. Ten % BAL in oil[3] and pitressin were administered by intramuscular injection.

Creatinine was measured by a modified Folin procedure (3), p-amino hippurate by the Bratton and Marshall reaction (4) and chlorides by a modification of the Volhard method (5).

Results. 1. *Effect of BAL on water and mercupurin diuresis.* The effect of an intramuscular injection of 1 ml. 10% BAL in oil on the diuresis and chloruresis resulting from the oral administration of 20 ml. water per kilogram body weight and 1 ml. mercupurin by vein was examined in each of 3 dogs. When

[1] This investigation was aided in part by a grant from the Carnegie Corporation of New York.

[2] Portions of this work were presented before the Eastern Section of the American Federation for Clinical Research, December 14, 1946.

[3] The authors are indebted to Lieutenant Colonel William T. DeVan of the Chemical Warfare Service, Edgewood Arsenal, Md., and to Dr. John H. Brewer of Hynson, Westcott and Dunning for a generous supply of 10% BAL in oil.

BAL was given to *dog A* 20 minutes before mercupurin, the maximum urine flow during the first hour was 1.1 ml. per minute and the maximum chloride excretion 4.4 microequivalents per minute, as compared to 5.8 ml. and 520 microequivalents per minute during the control experiment with mercupurin alone (fig. 1, C and D). In the BAL and mercupurin experiment there was a subsequent increase in urine flow, although chloride excretion remained at approximately the same level as was observed in the water-control experiment in this dog (fig. 1, A). The administration of BAL alone (fig. 1, B) resulted in a transient antidiuresis.

Similar effects were observed in *dog B* (fig. 2), although in this instance BAL was administered 15 minutes after the mercupurin. Toward the end of this experiment (fig. 2, D) there was a transient chloruresis, suggesting that there was not enough BAL to inactivate the mercurial completely.

BAL was administered at the height of diuresis in *dog C*, 40 minutes after the dose of mercupurin. Again, BAL inhibited the chloruretic effect of mercupurin

TABLE 1. *Protocol of typical experiment—dog A*

20 ml. of water per kgm. were given by stomach tube at 0 time

TIME	URINE FLOW	URINE CHLORIDE	GLOMERULAR FILTRATION	RENAL PLASMA FLOW	PLASMA CHLORIDE	HEMATOCRIT
min.	*ml./min.*	*μeq./min.*	*ml./min.*	*ml./min.*	*m.eq./l.*	*% RBC*
25– 45	0.60	4.1	—	—	108.5	57
45– 65	1.15	2.0	71	—		
65– 85	1.15	3.0	76	144		
85–105	2.00	4.0	74	148	105.8	
105–125	2.60	3.0	71	151		
125–145	2.95	3.3	72	133		
145–165	2.85	3.6	71	135	107.0	53

and caused a transient interruption in the diuresis resulting from the administration of water.

One-tenth ml. BAL appeared to be as effective in inhibiting the chloruresis following mercupurin as 1 ml. had been in *dog C*. However, administration of 0.05 ml. BAL to *dog A*, 35 minutes after mercupurin, had no significant effect on diuresis and only a partial and transient effect on the excretion of chloride.

2. *Effect of pitressin on water and mercupurin diuresis.* In view of the antidiuretic effect of BAL, pitressin, a preparation containing an antidiuretic hormone, was administered to the three dogs in experiments similar in design to the BAL studies. These data are shown in figure 4. The intramuscular injection of 10 units of pitressin in *dog B* prevented the diuresis expected from the administration of 20 ml. of water per kilogram body weight. Unlike BAL, this

[4] The authors wish to thank Dr. Richard de Bodo of the Department of Pharmacology of the New York University College of Medicine for his assistance and for his permission to use two of his dogs with permanent diabetes insipidus due to hypothalamic injury. The average daily fluid exchanges of these dogs prior to these studies were 3.0 and 3.5 liters, respectively.

dosage of pitressin resulted in a moderate chloruresis. The effect of small doses of pitressin to *dogs A and C*, however, was not much different from that of BAL (Compare to fig. 1B and 3B). The administration of 10 units of pitressin 35 minutes after mercupurin to *dog B* (fig. 4) inhibited diuresis, but chloride excretion rose to a high level.

3. *Effect of BAL on water diuresis in dogs with permanent diabetes insipidus.* One ml. 10% BAL in oil was injected intramuscularly into two dogs with diabetes insipidus[4] one hour after the administration of 40 ml. water per kilogram body weight. The rate of urine flow continued to rise after the BAL injection in each instance. The rates of glomerular filtration and chloride excretion were not measured in these experiments.

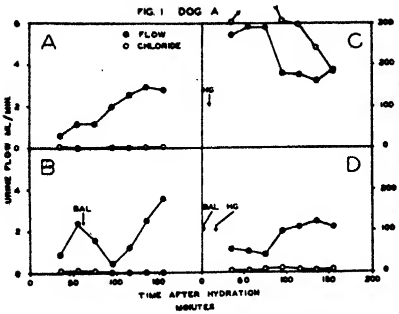
4. *Effect of BAL and pitressin on chloride excretion.* To augment chloride excretion, these experiments were initiated by the administration of 20 ml. 0.9% sodium chloride solution per kilogram body weight through a stomach tube, but otherwise were similar in design to the previous studies. The effect of injection of 1 ml. BAL in oil at the height of diuresis in two experiments on *dog B* (fig. 5) and in one experiment on *dog A*, as well as the effect of the administration of 0.2 and 0.025 units of pitressin to *dog B* at the height of saline diuresis, were examined. The data for some of the experiments on *dog B* are summarized in figure 5. Although in each instance there was a decrease in the rates of water and chloride excretion after the injections, there were no significant differences from the control saline experiment (fig. 5A).

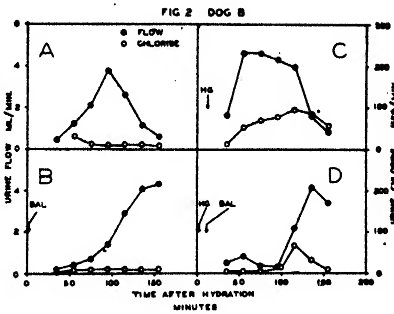
5. *Glomerular filtration rate, renal plasma flow, plasma chlorides and hematocrit.* Glomerular filtration rate was measured in all experiments except the two on dogs with diabetes insipidus. There were no trends in filtration rate that could be correlated with the effects of BAL or pitressin on the rates of water or chloride excretion. In *dogs A* and *B*, the filtration rates were consistently but only slightly increased during the second hour after fluid administration.

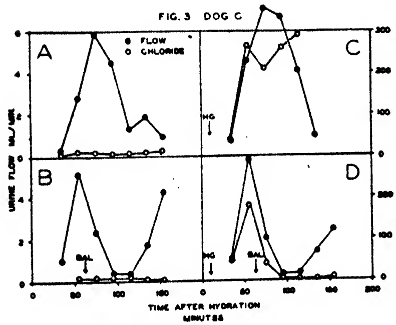
Renal plasma flow was measured during 11 experiments. This function was more variable than filtration rate, and the only regular trend observed was a 30% increase during the second hour after the administration of saline in 2 experiments on *dog A* and 1 on *dog B*.

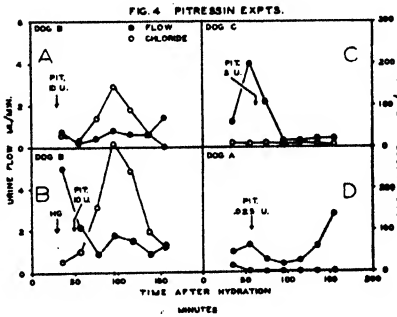
A fall in plasma chlorides was noted in 17 of the 18 experiments wherein 20 ml. water per kilogram body weight was given by mouth. The average fall for the entire group was 2.4 milliequivalents per liter of plasma. The hematocrit was followed serially in 9 of these experiments and fell an average of 7.2% after the water administration. In 7 experiments, 20 ml. of 0.9% sodium chloride solution was given per kilogram body weight. There was a slight fall in plasma chlorides in one, while the other 6 showed an average increase of 2 milliequivalents per liter of plasma. In this group of experiments, the average decrease in the hematocrit was 14.9%. The hematocrit changes quoted above should be interpreted with the knowledge that each experiment involved the withdrawal of 70 ml. whole blood.

6. *General reactions.* Adverse signs and symptoms were noted during only 3 of the 10 experiments when 1 ml. of BAL was administered. In each of these









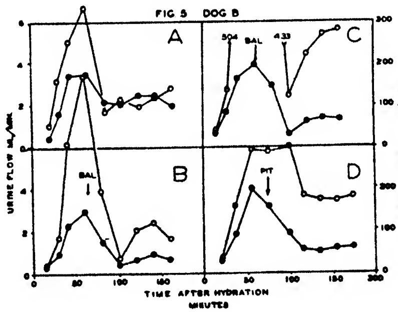


FIG. 1. Effect of mercupurin and BAL, alone and in combination, on water diuresis and chloride excretion in *dog A*. Each experiment began with the oral administration of 20 ml. water per kgm. body weight. *Dog A* weighs 23.6 kgm. ●——● = rate of urine flow. ○——○ = rate of chloride excretion. Hg = 1 ml. mercupurin intravenously. BAL = 1 ml. 10% BAL in oil intramuscularly.

FIG. 2. Effect of mercupurin and BAL, alone and in combination, on water diuresis and chloride excretion in *dog B*. Each experiment began with the oral administration of 20 ml. water per kgm. body weight. *Dog B* weighs 19.1 kgm. ●——● = rate of urine flow. ○——○ = rate of chloride excretion. Hg. = 1 ml. mercupurin intravenously. BAL = 1 ml. 10% BAL in oil intramuscularly.

FIG. 3. Effect of mercupurin and BAL, alone and in combination, on water diuresis and chloride excretion in *dog C*. Each experiment began with the oral administration of 20 ml. water per kgm. body weight. *Dog C* weighs 29.0 kgm. ●——● = rate of urine flow. ○——○ = rate of chloride excretion. Hg. = 1 ml. mercupurin intravenously. BAL = 1 ml. 10% BAL in oil intramuscularly.

FIG. 4. Effect of pitressin on water and mercupurin diuresis and chloruresis. Each

3 instances the animals vomited. *Dog B* vomited 34 minutes after 1 ml. of BAL had been given during the experiment shown in figure. 2B. *Dog B* also vomited 11 and 17 minutes after BAL during one experiment that was begun with the administration of saline, and again, 30 minutes after BAL in the other similar experiment.

DISCUSSION. There is little doubt, from the data presented above, that BAL can either prevent or promptly interrupt the diuretic effect of an organic mercurial. Recently it has been reported that BAL also prevents mersalyl diuresis in rabbits (9) and in dogs (10). The effect of BAL on mercupurin diuresis is two-fold. One ml. of 10% BAL in oil (100 mgm. BAL) administered intramuscularly is sufficient to eliminate completely (but not always permanently) the chloruretic effect of 1 ml. mercupurin (39.6 mgm. mercury) given intravenously. Urine flow is also markedly inhibited by the administration of BAL, but this effect is independent of any reaction with mercupurin, since BAL inhibits simple water diuresis, a mechanism probably mediated through the pituitary (see below).

No attempt was made to quantify the effect of BAL on mercupurin action. However, it appears that the technique used in these experiments, i.e., effect of BAL on chloruretic action of mercury, could be used as a simple, rapid and sensitive assay for compounds of the BAL type.

It is generally believed that heavy metals, including mercury, affect biological systems by combining with sulfhydryl groups of the protein portions of enzymes to form mercaptides (6, 7, 8). If this be the case, it seems likely that mercury exerts its diuretic effect in this manner as well (11). Indeed, it has been shown that the most effective organic mercurial diuretics are those which are the most readily ionizable (12). The fact that BAL can interrupt mercupurin chloruresis is further evidence that the mercury ion is the active principle of organic mercurial diuretics, at least so far as their effects on electrolyte excretion are concerned. It is unlikely that BAL can react with un-ionized mercupurin.

The antidiuretic effect of BAL is striking but transient. Water diuresis is inhibited within 15 minutes after the injection of BAL, the rate of urine flow usually falling to the range of 0.2 to 0.4 ml. per minute. In several instances the antidiuretic effect of BAL was preceded by vomiting. Vomiting, however, is not a prerequisite for the antidiuretic action.

It seemed possible that BAL might stimulate the secretion of the antidiuretic hormone of the posterior pituitary. Two types of experiments were set up to examine this possibility. The data summarized in figure 4 indicate that the effects on water diuresis of the proper intramuscular dose of pitressin, a posterior pituitary preparation containing an antidiuretic hormone, are quite similar to those of BAL. More direct evidence that BAL does exert its antidiuretic action

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experiment began with the oral administration of 20 ml. water per kgm. body weight. ●——● = rate of urine flow. ○——○ = rate of chloride excretion. Hg. = 1 ml. mercupurin intravenously. Pit = pitressin intramuscularly. U = units of pitressin.

FIG. 5. Effect of BAL and pitressin on chloride excretion. Each experiment began with the oral administration of 20 ml. per kgm. of 0.9% NaCl solution. ●——● = rate of urine flow. ○——○ = rate of chloride excretion. BAL = 1 ml. 10% BAL in oil intramuscularly. Pit = 0.2 units pitressin intramuscularly.

through the posterior pituitary was obtained in experiments on dogs with permanent diabetes insipidus. In these animals, BAL exerted no antidiuretic action.

That the effect of BAL on the chloruresis of mercupurin is not due to the posterior pituitary antidiuretic hormone is suggested by the experiment summarized in the lower left-hand corner of figure 4. Pitressin inhibited the diuresis in the mercupurin experiment, but chloride excretion reached a very high level in marked contrast to the effect of BAL in a similar circumstance.[5]

The lack of correlation between glomerular filtration rate and the various effects of mercupurin, BAL, and pitressin on diuresis and chloruresis indicate that the phenomena described in this report are the result of actions on the renal tubules. Evidence of hemodilution was obtained in each experiment by observation of decreases of both plasma chlorides and the hematocrit in the experiments initiated with water as well as the hematocrit in the experiments initiated with saline solution. It is likely, therefore, that the fluids administered by mouth were well absorbed, and that changes in urine flow were not due to variations in this function.

SUMMARY AND CONCLUSIONS

1. 2,3-dimercaptopropanol (BAL) can prevent or promptly interrupt the diuresis and chloruresis resulting from the intravenous administration of mercupurin.
2. BAL inhibits water diuresis.
3. BAL probably inhibits the chloruretic effect of mercupurin by combining with the mercury.
4. BAL probably inhibits water diuresis by stimulating the secretion of the antidiuretic hormone of the posterior pituitary.

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[5] A chloruretic factor, probably present in large doses of pitressin (10 units in this experiment), may have contributed in part to the great increase observed in the rate of chloride excretion.

EFFECT OF LANATOSIDE C UPON THE SURVIVAL OF RATS SUBJECTED TO SEVERE HYPOTHERMIA[1]

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Received for publication August 19, 1947

The circulatory failure in rats subjected to marked reduction of their body temperature has been reported in a previous communication (1). The present series of experiments was undertaken in order to study the effects of a pure digitalis glycoside upon the functional changes in hypothermia which lead to circulatory collapse.

The usual course of events in death from hypothermia, i.e., the arrest of respiration before the cessation of heart beats, has led most investigators to ascribe death to respiratory failure (2–5). However, Woodruff (6) reported that circulatory failure appeared to be the cause of death in some of the dogs rendered hypothermic in his experiments. Digitalis was effective in preventing complete circulatory collapse in some animals which showed signs of circulatory failure during cooling, but none of the human victims of German experiments on hypothermia responded favorably to intravenous injections of strophanthin (7). Attention was drawn by Talbott (8) to the rôle of the circulation in fatal hypothermia. He stated that " . . . most patients who die after exposure suffer from terminal cardiac failure."

If the failure of respiration in hypothermia described by many investigators (2–5) is brought about by a direct influence of low temperature (cf. Britton, 4), improvement of the circulation alone would not prolong the survival of animals cooled to body temperatures which are usually fatal. On the other hand, if failing circulation is the primary factor leading to depression and final failure of the respiration, then animals in which failure of the circulation is prevented or postponed should survive the reduction of their body temperature to levels lower than those tolerated by untreated animals. In addition, the improved circulation should tend to prevent the onset of the usual signs of cardiac asphyxia which are encountered in severe hypothermia (1, 9).

The data reported here include observations of respiration, heart rate, arterial pressure and the electrocardiogram of rats during induced hypothermia and the modifications of the response to cooling produced by the intravenous injection of a pure digitalis glycoside.[3]

[1] Supported by grants from the John and Mary R. Markle Foundation and from Medical Research Funds of the Stanford University School of Medicine.

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[3] Lanatoside, C, as Cedilanid, was generously supplied by Mr. Harry Althouse of the Sandoz Chemical Co.

Methods. The methods employed for the induction of hypothermia in rats and the measurement of heart rate, arterial pressure and the electrocardiogram have been described elsewhere (1).

Animals used in this study were adult male rats of the Slonaker-Wistar strain. Both controls and those receiving lanatoside C were anesthetized with 37.5 mgm. per kilogram of body weight of pentobarbital given intraperitoneally. The induction of hypothermia was begun in control animals after the usual interval of 90 minutes from the time of injection of anesthetic, while those in the treated group were given lanatoside C via the exposed saphenous vein of the right leg as soon as the anesthetic had taken full effect. The glycoside was given in 0.2% solution in doses of 1.12 to 1.41 mgm. per kilogram of body weight. Twenty minutes after the injection of lanatoside C, the initial measurement of respiration rate, electrocardiographic response and arterial pressure were begun. Induction of hypothermia then followed at 90 minutes from the time of anesthesia. Of 13

Table 1. *The effect of lanatoside C upon the lethal body temperature in hypothermia as indicated by the number of rats living or dead at 2-degree intervals from 21°C. down to 7°C. rectal temperature*

TEMPERATURE	CONTROLS		LANATOSIDE C	
	No. living	No. dead	No. living	No. dead
deg. C.				
21	11	0	13	0
19	11	0	13	0
17	9	2	13	0
15	5	6	13	0
13	1	10	9	4
11	0	11	3	10
9	0	11	2	11
7	0	11	0	13

animals treated with lanatoside C 5 were used for measurement of arterial pressure as well as respiratory rates, heart rates and electrocardiographic changes. Arterial pressure measurements were omitted on the remaining 8 rats.

Results. The general response of the glycoside-treated rats to hypothermia indicated an improved ability to withstand the effects of lowered body temperature. Shivering was observed in some of the treated animals at rectal temperatures as low as 14.5°C., while in the controls the lowest temperature at which shivering was observed was 16°C. The treated animals, which were not allowed to recover, died at rectal temperatures about 2°C. to 5°C. below the rectal temperatures at which death occurred in the controls. Table 1 presents the relative numbers of animals living and dead in the two groups over the range of temperatures from 21° down to 7°C. Probit analysis of these data indicated that the difference between the treated and untreated groups is significant: $P < 0.01$ (10).

Final cardiac arrest was preceded by respiratory failure in the treated animals as it was in the controls. Examination of the viscera post mortem showed the

same signs of congestive cardiac failure as were seen in the controls, except that the pulmonary engorgement was less marked and pulmonary edema was rarely encountered.

The depression of respiration which occurs during hypothermia was notably less severe in glycoside-treated animals than in the controls. Respiration not only persisted to lower body temperatures, but the rate was maintained at higher levels in the critical range below 20°C. Respiratory rates in the treated and control series were compared at intervals of 2 degrees over the temperature range from 36° down to 14°C. Statistical evaluation of the difference between the two

TABLE 2. *Mean values and standard errors of respiratory rates determined on digitalized and control rats over 2-degree intervals for the temperature range 14–36°C.*

	TEMP. INTERVALS										
	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36
Controls											
No. of readings	6	26	38	37	32	28	29	27	22	22	7
Mean resp. rate	4	5	10	22	35	47	54	67	70	66	70
S.E. of mean	1.4	1.0	6.4	2.1	2.6	2.6	2.2	2.4	2.7	2.6	6.4
Digitalized											
No. of readings	13	14	15	9	12	14	13	13	13	21	8
Mean resp. rate	16	17	23	36	51	61	65	70	91	90	88
S.E. of mean	3.2	3.5	3.3	5.4	4.2	4.1	3.3	3.2	4.7	3.7	4.0

$S.E._{\bar{d}}$: = 0.30. $P<0.001$.

The following formulae were used in the calculation of significance of difference between two series of paired means by the method of Lundborg and Wahlund (16).:

$$\bar{d} = \frac{1}{N}\sum_{1}^{N}\left[\frac{\bar{x}_i - \bar{y}_i}{S(\bar{x}_i - \bar{y}_i)}\right];\ S.E._{\bar{d}} = \frac{1}{\sqrt{N}};\ t = \frac{\bar{d} - 0}{\frac{1}{\sqrt{N}}} = \bar{d}\sqrt{N}$$

$\bar{d}$ = Average difference between 2 series; N = number of pairs; $\bar{x}_i$ & $\bar{y}_i$ = corresponding pairs.

series by the method of Lundborg and Wahlund (11) yielded a value for $P < 0.001$, or less than one chance in one thousand that such a difference would be encountered in similar series through errors of random sampling. A similar value for P was obtained by the method of Fisher (12). Since the comparison involves only living animals of both series, errors of sampling attributable to death of control rats at the low temperatures do not impair the validity. Table 2 gives values for standard errors of mean respiratory rate for each 2-degree temperature interval.

The decline of heart rate with falling body temperature in animals cooled after the injection of lanatoside C was the same as that observed in the controls. Electrocardiograms taken on treated, anesthetized animals which were not subjected to hypothermia showed some slowing of the heart rate, but no consistent changes were observed in the contour of the waves nor in A-V conduction time.

Serial electrocardiograms taken on treated rats which were subjected to hypothermia showed no differences from those taken on controls until the rectal temperature fell below 20°C. At rectal temperatures between 20° and 15°C., arrhythmias of sinus origin were frequently seen. Disturbances of A-V or intraventricular conduction were encountered less often than in the controls, and, when they did appear, they tended to be transient, while in the controls S-A or A-V block became well established (1). The most striking feature of the electrocardiograms taken on treated, cooled rats at rectal temperatures below 15°C. was the appearance of long periods of complete cardiac arrest, lasting in

TABLE 3. *Mean values, standard errors of means and significance of difference between means (expressed as P) of arterial pressure measurements determined on digitalized and control rats over 2-degree intervals for the rectal temperature range 14°–36°C.*

	TEMP. INTERVALS										
	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36
Controls											
No. of readings...	15	27	43	35	25	23	22	16	18	8	9
Mean A.P. mm. Hg...........	42	56	81	105	124	131	137	131	135	132	142
S.E. of mean......	10.9	7.2	5.8	6.4	4.2	4.5	4.2	3.8	4.8	5.4	5.4
Digitalized											
No. of readings...	16	12	11	8	9	7	5	9	7	6	5
Mean A.P. mm. Hg...........	48	92	111	122	138	137	144	138	140	138	150
S.E. of mean......	7.0	5.3	4.0	3.4	3.6	3.6	3.2	3.4	4.1	6.3	9.2
P[1]...............	.6 .5	.001	.001	.01 .001	.01 .001	.2 .1	.2 .1	.1 .05	.3 .2	.4 .3	.4 .3

[1] Where 2 values are given for *P*, the actual value for *P* lies between the extremes given. The *P* value for the difference between the two series taken as a whole was <0.01 (12).

some cases 60 to 90 seconds, followed by the sudden reestablishment of regular cardiac cycles without abnormalities of conduction.

The data in table 3 show the effect of reduction of body temperature upon the arterial pressure in animals receiving lanatoside C and in controls. The critical decline of arterial pressure was delayed in the treated group until the rectal temperature had fallen about 2°C. below the temperature associated with a similar decline in the controls. Over the range of maximal change the mean of the arterial pressures was higher at any given rectal temperature in the treated group that in the controls. Statistical comparison of the pairs of mean arterial pressures at the various 2-degree intervals given in table 3 showed that significant differences are encountered over the critical temperature range between 16° and 24°C. Arterial pressures from 25 to 70 mm. Hg or more were measured in the treated animals at rectal temperature levels not survived by any of the controls.

Comparison of the two series in their entirety by the method of Fisher (12) yielded a value for P of < 0.01.

The relationship between arterial pressure and heart rate of control rats and those treated with glycoside is shown in figure 1. Neither the controls nor the treated animals showed any signs of decreased peripheral resistance such as diminution of skin pallor during the period of hypothermia. The assumption that the decline in arterial pressure with decreasing rectal temperature, especially from about 25°C. down to termination, was the result of reduced cardiac output is in accord with the above observation and with the changes in venous pressure reported below. The differences indicated by the plotted mean values for controls and treated animals in figure 1 were not found to be statistically significant

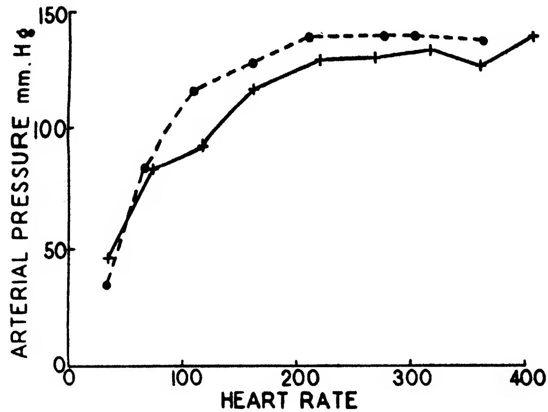

FIG. 1. Relationship between arterial pressure and heart rate during hypothermia in animals treated with 1.12 to 1.41 mgm/kgm. of lanatoside C and in untreated controls. Crosses—11 controls; dots—5 treated animals.

over the series as a whole. However, a highly significant difference was demonstrated between the two groups when the heart rates had declined to between 100 and 150 beats per minute and the rectal temperatures were 18° to 22°C. Application of 'student's' t test (12) for the significance of difference to measurements made on 21 control and 5 treated rats gave, with 37 degrees of freedom, a value for t of 5.0648, and P was less than 0.001.

The relationship between shivering, respiration and venous pressure in untreated rats during hypothermia is illustrated in table 4. The data include observations of venous pressure and rectal temperature before the onset of shivering, at the maximum of shivering and after the cessation of shivering. Venous pressure was measured in the external jugular vein just before the vein entered the thorax. The method of reading the pressures was a modification of that used by Wells, Youmans and Miller (13) for measurement of interstitial fluid pressure and involved the introduction into the blood stream of only a few cubic millimeters of Ringer's solution at each determination.

In all of the animals presented in table 4 the venous pressure rose during maxi-

mum shivering and, in all but one animal, underwent a marked decrease with the cessation of shivering. It is suggested, therefore, that one of the primary events leading to reduction of cardiac output in the range of body temperature from 24° down to 16°C. is the progressive reduction of ventricular filling during diastole.

DISCUSSION. The experiments presented above show that rats receiving intravenous injections of lanatoside C are able to withstand reduction of their body temperature to lower levels than untreated controls. The evidence for the greater resistance of the animals to the deleterious effects of cold was the maintenance of both shivering and spontaneous respiration in treated animals at body temperatures which were associated with complete cessation of these processes in untreated controls and the reduction of minimum lethal temperature by 2° to 5°C.

TABLE 4. *Venous pressure, respiratory rate and rectal temperature in relation to shivering during hypothermia; data from 5 animals*

INTERVAL	MEASUREMENT	RAT NUMBER				
		1	2	3	4	5
Before shivering	Venous pressure, mm. H_2O	+30	+9	−15	+62	−11
	Respirations per minute	66	90	72	66	66
	Rectal temperature, °C.	34.5	35.0	35.9	35.5	37.6
Maximal shivering	Venous pressure, mm. H_2O	+44	+34	−3	+113	+55
	Respirations per minute	96	120	40	72	54
	Rectal temperature, °C.	30.0	31.2	25.0	31.2	25.3
Cessation of shivering	Venous pressure, mm. H_2O	+13	−7	+3	+31	+7
	Respirations per minute	24	30	10	2	10
	Rectal temperature, °C.	15.9	20.8	19.2	17.0	16.0

The data do not permit quantitative analysis of the factors which are responsible for the decreased cardiac output observed in both series. Figure 1 shows that the decline in heart rate did not become associated with a serious fall in arterial pressure until the rate had decreased below 150 beats per minute. With further reduction of heart rate as the body temperature was lowered, the decrease in arterial pressure became more marked. Certain associated phenomena such as increased respiration and shivering served to aid venous return to the heart during the phase of cooling when the rectal temperature was above 24°C. With further cooling the reduced intensity and final cessation of shivering and the slower, shallower respiration at the lower temperatures tended to remove important aids to diastolic filling. Thus the more prolonged period of shivering and more effective respiration observed in the glycoside-treated animals should be expected to contribute to the maintenance of cardiac output at a higher level in these animals. The above mechanisms of improved cardiac output should be considered as supplementary to the direct action of digitalis glycoside upon dilated hearts (14).

These observations show that at least two aspects of central nervous system function, *a*) the discharges producing shivering and *b*) the rhythmic activity of the respiratory center, persist in spite of the reduction of body temperature substantially below the levels associated with their disappearance in untreated rats. Over the ranges of body temperature within which the disappearance of shivering and the failure of respiration occur in untreated animals, the measurements of arterial pressure indicate profound circulatory collapse, while similar measurements upon rats treated with lanatoside C yield mean arterial pressure values of 70 mm. Hg or more. The manifestations of cardiac anoxia which are a common feature of electrocardiograms taken on untreated, hypothermic rats are observed only as terminal events and at appreciably lower body temperatures in rats injected with lanatoside C. Thus the higher perfusion pressure appears to protect both the central nervous system and the heart muscle. Since measurements of brain temperature and heart temperature show that these regions are not significantly warmer than the rectum (15), the improved function may not be ascribed to local increases in temperature. Impairment of function during anoxia has been shown to occur in both the respiratory center (16) and in the heart (17), but both brain tissue (18) and heart muscle (19) studied *in vitro* have been shown to recover their original rate of oxygen consumption at 37°C. after being maintained for one hour at less than 1°C. It should be pointed out that the gas phase in the system used for study of the isolated tissues was pure oxygen, and thus the situation cannot be considered comparable with that encountered in the intact, hypothermic animal where the depressing effect of low temperature upon metabolism is complicated by asphyxia as the result of progressive circulatory failure. The improvement in function of the heart and the central nervous system of the animals in severe hypothermia after treatment with lanatoside C was evident only during the period when the mean arterial pressure was held above 50 mm. Hg. Thus the maintenance of function in the 'supply mechanisms' as studied in these experiments appears to be relatively independent of temperature and relatively dependent upon the level of perfusion pressure in the circulation.

SUMMARY

1. The changes in heart rate, respiratory rate, the arterial pressure and the electrocardiogram during hypothermia were studied in rats treated with intravenous injections of lanatoside C and untreated controls.

2. The following signs of improved ability to withstand the effects of lowered body temperature were observed in the treated animals:

a. Shivering persisted down to rectal temperatures about 1.5°C. below the lowest temperature at which shivering was observed in the controls.

b. The minimum lethal temperature was 2 to 5°C. lower for the treated rats than that for the controls.

c. Respiratory rate was better maintained at low rectal temperatures in the treated animals than in the controls.

d. Terminal pulmonary edema was observed frequently in untreated controls, but those receiving lanatoside C rarely developed pulmonary edema.

3. Hypothermic rats treated with lanatoside C showed fewer disturbances of rhythm and conduction, as judged from electrocardiographic examination, than untreated, hypothermic animals.

4. The critical decline of arterial pressure during hypothermia did not appear in rats treated with pure digitalis glycoside until the rectal temperature had fallen about 2°C. below the level at which a similar decline occurred in the controls.

5. Sustained arterial pressure with declining heart rate in hypothermic animals treated with digitalis glycoside depended in part upon the longer persistence of shivering and adequate respiratory movements as factors in venous return and in part upon the direct cardiac action of the glycoside.

6. Respiratory failure in hypothermia is relatively independent of the body temperature and relatively dependent upon the level of perfusion pressure in the circulation.

The authors wish to thank Dr. F. W. Weymouth for carrying out the probit analysis included in this report as well as for general advice upon the statistical treatment of the data.

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EXCRETION OF THE BLUE DYE, T-1824, IN THE BILE

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Received for publication September 16, 1947

The ultimate fate of intravenously injected T-1824 has not been established. It is known to be bound firmly to plasma albumin (1) and hence to appear in the urine only when glomerular damage is present. By analogy with other vital dyes (2), it has been presumed that T-1824 is excreted in the bile. This has been verified qualitatively (3), but no quantitative data have been reported. The present study was undertaken in the hope that accurate data on the disposition of the dye after its escape from the blood stream might aid in the interpretation of the dye disappearance curve as it is used in plasma volume determinations.

EXPERIMENTAL. *Analysis of dye in bile.* The spectrophotometric determination of the dye in the presence of bile pigments presented certain difficulties. Preliminary studies indicated that the concentration of bile pigment in successive samples of hepatic bile is usually quite variable, so that a dye-free bile blank could not be used. Attempts to separate the dye from bile pigment by extraction with organic solvents, differential adsorption or dialysis through cellophane membranes were not quantitatively successful. Complete spectral absorption studies on the bile pigments, both in the naturally occurring forms and when completely converted either to bilirubin or to biliverdin, revealed that the bile pigments absorb strongly at all wavelengths at which there is absorption by the dye, so that the usual method for the determination of one colored substance in the presence of another could not be used. A modification of this principle was finally adopted. At 610 mμ, the absorption peak for the dye in bile, there is strong absorption by bile pigment, but at 750 mμ, absorption by the dye becomes negligible while absorption by bile pigment remains appreciable. If concentration-transmission curves of bile pigment at 610 and 750 mμ are established, the absorption by bile pigment at 610 mμ can be estimated from its absorption at 750 mμ, even though dye is also present (see fig. 1). The total absorption at 610 mμ is a function (not the arithmetic sum) of the separate absorptions by dye and bile pigment. If the total absorption at 610 mμ and the estimated bile-pigment absorption at 610 mμ are converted to equivalent dye concentrations by reference to a concentration-transmission curve of dye in bile, the actual dye concentration is obtained by difference. The validity of this procedure was verified by analysis of bile samples to which known amounts of dye had been added.

A potential source of error is the rapid but variable conversion of bilirubin to biliverdin on exposure to air, with a resulting change in the optical properties of the pigment. To eliminate this difficulty the following procedure was adopted. The bile samples were collected under oil. An aliquot of each sample was diluted to the desired volume with oxygen-free saline, layered with melted paraffin and

analyzed within 5 minutes. Control experiments demonstrated the adequacy of this procedure.

Plan of experiments. Dogs anesthetized with sodium pentobarbital were used. The cystic duct was ligated and the common bile duct cannulated for continuous collection of hepatic bile. The dead space of the cannula was reduced to a minimum in order to lessen the delay between the secretion of bile and its collection. In one experiment thoracic duct lymph was also collected by cannula. Four or five 30-minute samples of bile were obtained to permit the calculation of the relation of the spectral absorptions of bile pigment at 610 and 750 mμ, since this varied in different experiments. Ten milligrams of T-1824 was then injected into a jugular vein and serial blood samples were drawn from another vein for determination of the plasma dye concentration corresponding to that of each bile sample. Each experiment was continued until it was certain that the concentration of dye in the bile had reached a maximum and had begun to decline.

Results. Nine technically satisfactory experiments were performed including one in which the dye concentration in thoracic duct lymph was also determined. In this series of experiments the rate of hepatic bile flow ranged from 2 to 10 ml. per hour and the plasma dye concentration ranged from 2 to 34 mgm. %.

The latent period from the injection of dye until its appearance in the bile was difficult to estimate with accuracy. The bile collected during the first 30 minutes following injection of dye contained very small amounts of the dye. Thereafter the concentration rose rapidly, reaching a maximum 60 to 90 minutes following injection and then declining at a rate roughly proportional to the simultaneous decline in plasma dye concentration (fig. 2)

The concentration of dye in the bile was always lower than (usually less than one-half) the simultaneous plasma dye concentration and roughly proportional to it. There was no obvious relation between the concentration of dye in the bile and the rate of bile flow. In the experiment in which thoracic duct lymph was collected the dye appeared in the lymph more promptly and in higher concentration than in the bile (fig. 3).

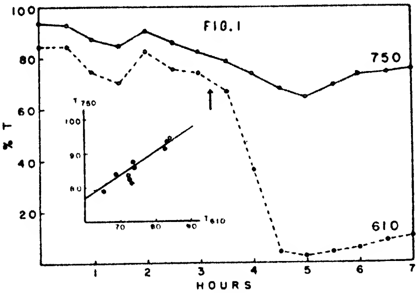
The relatively low concentration of the dye in the bile might indicate either that the dye is filtered through a membrane system with a low permeability or that it is secreted by a mechanism having a low maximal capacity. In order to obtain further information on this point, very large amounts of dye were injected in two experiments (60 and 70 mgm., respectively, in dogs weighing 9.6 and 11.4 kgm.). When the concentration of dye in the bile had reached a maximum and had begun to fall, a second injection of dye, in amount equivalent to that of the first injection, was made. In both experiments the concentration of dye in the bile increased again, proving that the maximal concentration of dye in the bile following the first injection was not limited by saturation of a secretory mechanism.

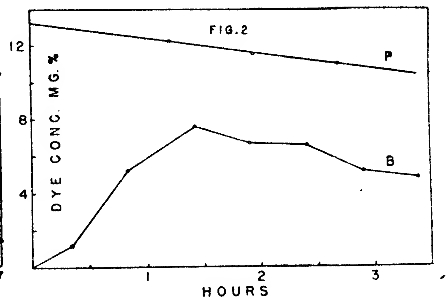
Strips of cellophane soaked in dyed hepatic bile were stained a deep purple. When these strips were washed in running water for 24 hours, the purple color was removed and a permanent blue staining of the cellophane was revealed.

This is presumptive evidence that some, if not all, of the dye appearing in the bile is not bound by protein, since cellophane soaked in dyed plasma is not stained.

In several experiments the feces of animals receiving dye intravenously or by stomach tube were analyzed, but no dye could be detected.

DISCUSSION. Intravenously injected substances escape from the blood stream at varying rates and by a number of channels. The selection of a suitable substance for determination of plasma volume by the dilution method is influenced by both the method and the rate of its removal from the circulation. The blue





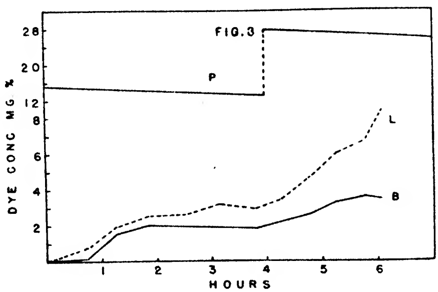


FIG. 1. Light transmittance of serial samples of hepatic bile at 610 and 750 mμ. T-1824 injected at arrow. *Insert:* relation between light transmittance of undyed bile samples at 610 and 750 mμ.

FIG. 2. Simultaneous dye concentrations in blood plasma (P) and in hepatic bile (B). T-1824 injected at zero time.

FIG. 3. Time-concentration curves of T-1824 in plasma (P), thoracic duct lymph (L) and hepatic bile (B). T-1824 injected at zero time and at 4 hours.

dye T-1824 has been widely used for this purpose because of its slow rate of disappearance. It has been generally assumed that T-1824, by virtue of its binding to plasma protein, escapes only at the slow rate at which protein leaks through systemic capillaries. This assumption, which is basic in the determination of plasma volume by both the single sample and the extrapolation methods, has recently been challenged. It has been claimed (4) that a significant amount of the dye is removed by phagocytosis in the first few minutes following injection and that the rapid initial fall in dye concentration is due to this removal, not to mixing of the dye in the circulation. It has been further suggested that the liver

is importantly involved in this phagocytic process by virtue of its very active reticulo-endothelial system. Accordingly, one of the major objectives of the present study was an estimate of the relative importance of the liver in the removal of T-1824 from the blood stream.

In each experiment, the total amount of dye which appeared in the bile during the observation period of 2 to 5 hours was calculated from the volume and dye concentration of the serial bile samples. From the simultaneous plasma dye disappearance curve, the amount of dye lost from the circulation during the same period could be calculated. Of this amount an average of 4.1% (range 2.0 to 6.7) was accounted for by the dye excreted in the bile. It is apparent that, even though the bile may be the ultimate channel of excretion of intravenously injected T-1824, the liver does not remove a sufficient amount of dye during a given period to influence the slope of the dye disappearance curve significantly.

The proponents of the theory that phagocytosis is responsible for the rapid fall in plasma dye concentration in the first few minutes following injection suggest that this rapid fall is checked by 'saturation' of the reticulo-endothelial elements. The experiments reported here indicate that if the hepatic phagocytes do participate in the removal of dye from the blood, they are not saturated by the usual amounts of dye employed in plasma volume determinations, since a second injection of dye is followed by a proportionate rise in dye concentration in the bile.

These experiments do not rule out the possibility of phagocytosis of dye by the Kupffer cells of the liver. In fact, the staining of cellophane by dyed bile indicates that the dye-protein bond has been broken during the process of hepatic excretion and phagocytosis is the most reasonable mechanism involved. However, the fact that the rapid initial decline in plasma dye concentration is checked at a time at which the reticulo-endothelial cells are far from saturated mitigates against their importance in this process.

SUMMARY

The hepatic removal of intravenously injected T-1824 has been studied because of its bearing on the interpretation of the T-1824 disappearance curve. Spectrophotometric analyses of hepatic bile and of blood serum, supplemented by chemical separation of the dye from bile, permitted the following conclusions:

(1) Very little dye appears in hepatic bile in the first 30 minutes after injection.

(2) Maximum dye concentration in bile is reached 60 to 90 minutes after injection; dye concentration then declines slowly, paralleling the fall in plasma dye concentration.

(3) Dye concentration in the bile is always lower than (usually less than one-half) the simultaneous plasma dye concentration, but roughly proportional to it.

(4) The equilibrium dye concentration in bile is not due to the saturation of a secretory mechanism (as in renal Tm) because a second injection of dye results in a second rise in dye concentration in bile.

(5) The concentration of dye in bile bears no obvious relation to the rate of bile flow within the limits of flow rate encountered (2 to 10 ml. of bile per hour).

(6) The excretion of dye in the bile in the first 2 to 5 hours following injection

accounts for only 2 to 7% of the dye which leaves the blood stream in that interval. Hence liver function is presumably not a limiting factor in the rate of dye disappearance.

(7) The concentration of dye in bile parallels (but at a lower level) that of dye in thoracic duct lymph.

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A RE-EVALUATION OF THE T-1824 MIXING CURVE

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Received for publication September 16, 1947

The widespread use of dye methods for determining plasma volume has resulted in progressive refinements in technique but has left unsolved many fundamental issues concerning the proper interpretation of the results. Since a quantitative knowledge of the behavior of the dye in the body is essential to an intelligent use of the method, a comprehensive study of the blue dye T-1824, from the time of injection to its ultimate excretion, has been undertaken in this laboratory. This paper deals with the time required for mixing of the dye in both circulating and non-circulating plasma. Subsequent reports will consider the factors involved in the loss of dye from the bloodstream and its ultimate fate in the body

METHODS. The same lot of dye (Eastman Kodak Company, No. 3873) was used in all experiments. Spectral absorption studies indicated maximal light absorption by the dye in serum at 620 mμ. A stock solution of dye (0.1% in distilled water) was used for injection and the standard dose was 10 ml. of solution, regardless of the size of the animal. Fasting, unanesthetized dogs were used; they were tied to an animal board during sampling procedures and were replaced in a cage without access to water in the intervals between samples. The dye was injected into one jugular vein and blood samples were obtained, without stasis, from the other jugular vein. The blood was centrifuged under oil and the dyed serum, diluted with 4 volumes of saline, was read against a serum-saline blank in a Coleman spectrophotometer. Duplicate readings were required to check within 0.2%. Hemolysis was seldom encountered; when it was minimal (as indicated by a slight greenish color of the dyed serum) the method of correction suggested by Gibson and Evans (1) was employed. Samples showing more than minimal hemolysis were discarded. Time-concentration curves of the dye were established by plotting the logarithm of the dye concentration against time on large-scale coordinate paper.

RESULTS. The results of 105 experiments on 24 dogs are included in this report. Some of these experiments were part of another study and the invariable finding of a greater apparent rate of dye disappearance during the first hour after injection than during subsequent periods led to the present study. The data from these earlier experiments are included here to give greater weight to the quantitative conclusions drawn. In the later experiments, the timing of the blood samples was designed to give maximal information on breaks in the slope of the time-concentration curve; samples were taken at intervals of 1, 2, 5, 10, 20, 40 and 60 minutes and 2, 3, 4, 5, 6 and 7 hours after injection of the dye. The time-concentration curve from a typical experiment is reproduced in figure 1,

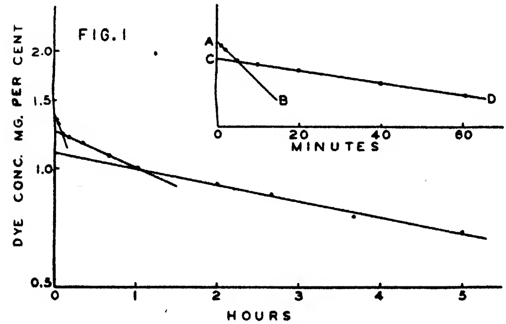
curve I. It is apparent that the mixing of the dye in the plasma is divisible into two phases based on the break which occurs in the mixing curve. Phase I, which has a slope equivalent to a dye loss of 80–100% per hour, is complete in 4 to 6 minutes (in rare cases, the duration of phase I is extended to 10 minutes). Phase II, which has a slope equivalent to a dye loss of 20–30% per hour, is usually complete in 30 to 50 minutes, with exceptional cases extending the limits to 20 to 60 minutes. In figure 1, curve II, the mixing curve only from another experiment is reproduced on an exaggerated time scale to differentiate phases I and II more distinctly.

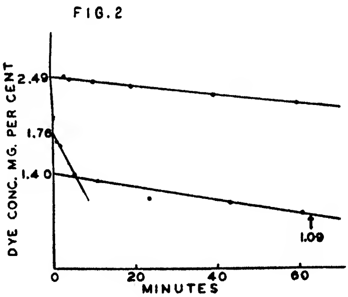
Since blood samples taken simultaneously from different regions of the circulation have identical dye concentrations within 10 minutes after injection of dye (2), it is a logical inference that phase I of the mixing curve represents mixing of the dye in the immediately available or circulating plasma. Additional support for this concept is found in the work of Hahn (3), who reported that the concentration of intravenously injected tagged erythrocytes was essentially the same 10 minutes and 1 to 3 days after injection.

This interpretation of phase I has been questioned recently by Cruickshank and Whitfield (4), who attribute the rapid fall in dye concentration to phagocytic removal of dye from the bloodstream. Their evidence for this is the fact that a previous injection of dye or of India ink reduces the rate of decrease of dye concentration during this phase, presumably due to reticulo-endothelial blockade. Accordingly, they advocate extrapolation of the phase I portion of the time-concentration curve in the calculation of plasma volume. We have repeated and extended their observations and have partially confirmed their results, but not their interpretation. In 6 experiments, 2 equal injections of dye were made one hour apart and all serum samples were read against the same dye-free blank. A typical experiment is illustrated in figure 2. Since the rapid initial fall in dye concentration is absent or reduced in the mixing curve of the second injection, there is no question as to the proper method of extrapolation. The initial dye concentration thus determined minus the residual dye present at the time of the second injection should equal the true initial dye concentration following the first injection. This permits a theoretically correct choice to be made between extrapolation of the phase I mixing curve (as advocated by Cruickshank and Whitfield) and of the phase II mixing curve as advocated by the author. In every case, extrapolation of the phase I curve gave an initial dye concentration considerably above the theoretical value, while extrapolation of the phase II curve gave an initial dye concentration practically identical with the theoretical value. The correct interpretation of Cruickshank and Whitfield's results is uncertain.

Phase II of the mixing curve is tentatively interpreted as representing the diffusion of dye into relatively non-circulating plasma. The break in the time-concentration curve which occurs at the end of phase II would then mark the transition from the mixing curve to the true disappearance curve. Since the latter reflects the net rate of loss of dye from the bloodstream, its extrapolation yields a value for initial dye concentration from which the total plasma volume

can be calculated. During phase II, two factors contribute to the progressive decrease of dye concentration in the plasma: diffusion of dye into non-circulating plasma and loss of dye from the bloodstream. Hence, extrapolation of the phase II curve will give a higher initial dye concentration, corresponding to mixing of the dye in the circulating plasma alone. The obvious implication of this interpretation is that the simultaneous extrapolation of the phase II mixing curve





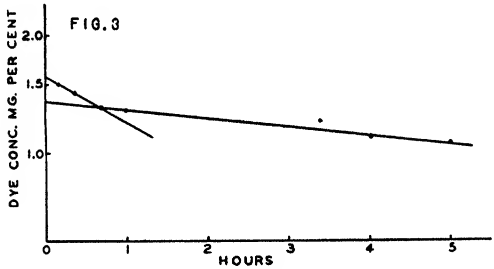


FIG. 1. Curve I (lower): Time-concentration curve of intravenously injected T-1824 showing the biphasic mixing curve and the true disappearance curve. Curve II (upper): The biphasic mixing curve; AB = phase I, CD = phase II.

FIG. 2. Time-concentration curves of double dye injections. Lower curve: initial injection; upper curve: second injection (made at point on lower time curve indicated by arrow). Residual dye concentration at time of second injection = 1.09 mgm. %.

FIG. 3. Time-concentration curve of T-1824 after ligation of thoracic duct and cervical lymphatic trunks. Note that the break in the curve at 40 minutes is unaltered by this procedure (see text).

and of the dye disappearance curve in the same animal permits the calculation of both total and circulating plasma volumes, which is highly important in many types of studies on the circulation. The validity of this procedure is indirectly confirmed by independent types of measurement, as noted in the discussion. In the experiment illustrated in figure 1, curve I, the values for total and circulating plasma volumes were 500 and 426 ml. respectively. In the complete series of animals, the average circulating plasma volume was 86% of the total plasma volume (range 84 to 91%).

The validity of this method of calculating total and circulating plasma volumes was tested by experiments designed to alter the relative proportions of circulating and non-circulating plasma. In 3 experiments adrenalin was administered by slow intravenous infusion for the purpose of diminishing the non-circulating plasma fraction. The circulating plasma volumes in these 3 experiments were 96, 93 and 97%, respectively, of the total plasma volume. In 2 experiments repeated injections of histamine were made in an attempt to increase the non-circulating fraction of the plasma volume. Circulating plasma volumes were 82 and 86%, respectively, of the total plasma volume.

The possibility that the break in the time-concentration curve, which marks the transition from the phase II mixing curve to the dye disappearance curve, might be due to the return of dyed lymph to the bloodstream has been investigated. In 3 animals, the thoracic duct and the cervical lymphatic trunk were ligated prior to injection of the dye. The time-concentration curve from a typical experiment is reproduced in figure 3. Prevention of the return of dyed lymph to the circulation does not alter the slope of any portion of the curve.

Discussion. Estimates of the time required for complete mixing of an injected dye in the circulation range from 90 seconds (5, 6) up to 20 minutes or longer (7). Such diversity of opinion indicates that the term 'mixing time' requires more precise definition, since it is inconceivable that such a range of values could be real, or due simply to differences in technique. This difficulty would seem to be resolved by subdivision of the mixing curve into two phases. The average time of 4 to 6 minutes required for mixing of the dye in the circulating plasma is consistent with evidence from other sources (2, 3). It is somewhat longer in human subjects (1) and varies inversely with the circulation velocity, being curtailed, for example, in hyperthyroidism and prolonged in congestive heart failure (1). These considerations suggest that the duration of phase I of the mixing curve might be used as a rough index of the over-all circulation velocity to supplement the pulmonary circulation velocity measured by such agents as cyanide and decholin.

The interpretation of phase II of the mixing curve as representing (in addition to loss of dye from the bloodstream) the diffusion of dye into non-circulating plasma is, of necessity, based on indirect evidence. In the first place, there is no general agreement on a precise definition of the term 'non-circulating plasma'. The experiments of Fåhraeus (8) have demonstrated beyond question the existence of peripheral layers of slowly moving or stagnant plasma in the 'small blood vessels'. The phenomena of plasma skimming (9) and of vasomotion (10) contribute variable volumes of presumably stagnant capillary plasma. Finally, variable fractions of the plasma in such organs as the liver and spleen probably should be added because of the anatomical peculiarities of the circulation in these regions. It is not intended that a sharp division between 'circulating' and 'non-circulating' plasma should be inferred from the form of the mixing curve, but rather a gradual transition between two constantly fluctuating fractions.

Indirect support for the idea that diffusion of dye into non-circulating plasma is the dominant factor in phase II of the mixing curve is derived from the fact that

calculations of the non-circulating fraction of the total plasma volume based on this assumption agree with those made by independent techniques. Thus, Smith, Arnold and Whipple (11) estimated, largely from anatomical considerations, that about ⅓ of the contents of the 'small blood vessels' represents stagnant plasma and that this is equivalent to approximately 15% of the total plasma volume. Hahn (3) calculated the fraction of plasma in 'relatively sluggish circulation' from experiments in which tagged erythrocytes were injected and obtained values ranging from 6 to 37%, with an average of 20.8%.

SUMMARY

(1) When the blue dye T-1824 is injected into the bloodstream of dogs, mixing of the dye in the plasma occurs in two phases. Phase I, which is completed 4 to 6 minutes after injection, represents mixing of the dye in the circulating plasma. Phase II, which is completed 30 to 50 minutes after injection, reflects the diffusion of the dye into non-circulating plasma.

(2) Extrapolation of the phase II portion of the mixing curve permits the calculation of circulating plasma volume, while extrapolation of the true disappearance curve (i.e., the time-concentration curve after completion of phase II) provides an index of total plasma volume. Based on this type of calculation, the circulating plasma volume in normal, unanesthetized dogs is approximately 86% of the total plasma volume.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 151 DECEMBER 1, 1947 No. 2

ENDOCRINE INFLUENCES ON CARDIAC OUTPUT AND OXYGEN CONSUMPTION IN DOGS[1]

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Received for publication September 12, 1947

We have reported (1) that the cardiac output of dogs is greatly reduced on hypophysectomy. This paper reports further cardiac output and oxygen consumption findings on simple hypophysectomy, on thyroidectomy, on combined hypophysectomy and thyroidectomy, on denervation of neural hypophysis, and on adminstration of anterior lobe extract.[2] The work has permitted comparisons with the effects of such procedures on renal blood flow (1, 6, 7, 8).

METHODS. The Fick method as described (1) was used for cardiac-output and oxygen-consumption determinations, the values per square meter of original surface area per minute being given; surface area in square meters = $0.2864\ W^{0.367}L$, where W is weight in kilograms and L is body length in meters, from tip of nose to root of tail (2). Dogs were kept on a uniform maintenance diet of Frisky chow biscuits daily and horse meat every other day; all determinations were made in the post-absorptive state and, except where otherwise designated, under sodium pentobarbital anesthesia, 30 milligrams per kilogram intravenously 30 to 45 minutes before determination. In most cases 3 preoperative runs were made.

Completeness of removal of thyroid was verified by careful inspection at autopsy; completeness of removal of anterior lobe in simple hypophysectomy was verified by brain sections and by examination of sella contents; denervation of neural hypophysis was shown by permanent polyuria.

RESULTS. *Thyroidectomy.* Figure 1 shows that daily subcutaneous administration of 225 units of preloban for 7 days to normal female *dog 11* somewhat more than doubled the cardiac output and almost doubled rate of oxygen consumption. Thyroidectomy significantly reduced both values in female *dogs 8* and *10*. Cardiac output before operation averaged 4.1 and 4.3 L/min/M² for *dogs 8* and *10*, respectively; 20 days after thyroidectomy the values were 3.2 and 2.8 or 78 and 65% of normal. Oxygen consumption before operation averaged

[1] This work was aided by a grant from the Commonwealth Fund.
[2] Preloban was given us by the Winthrop Chemical Company.

143 and 122 cc/min/M²; 20 days after operation it was 118 and 86 or 82 and 70% of normal.

Daily administration of 225 units of preloban for 6 days to thyroidectomized *dog 8* somewhat raised oxygen consumption, without effect on cardiac output; a subsequent 9-day period raised both values. Preloban for 9 days raised both values in thyroidectomized *dog 10*. In no case is the percentage effect of comparable preloban administration as great in the thyroidectomized as in the normal or the hypophysectomized dog, although in thyroidectomized *dog 10* it is almost as great.

Hypophysectomy. Figure 2 shows that simple hypophysectomy (removal of anterior lobe, pars intermedia and dependent portion of neural hypophysis) is

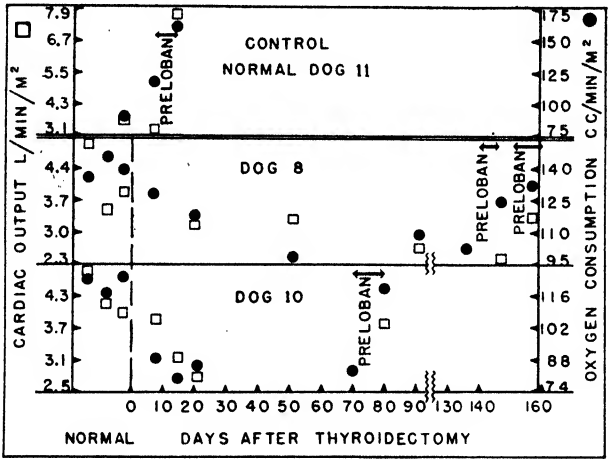

FIG. 1. Effects of preloban administration on cardiac output and oxygen consumption of normal *dog 11*; effect of thyroidectomy and of preloban administration to thyroidectomized *dogs 8* and *10*.

followed by a marked, prompt and permanent fall in cardiac output and in oxygen consumption. Subsequent thyroidectomy in *dogs 2* and *4* produced a slight further fall.

Daily 225-unit preloban administration for 7 days to hypophysectomized *dog 1* raised oxygen consumption without effect on cardiac output, while 8 days of daily 225-unit preloban administration raised both values to normal or above in hypophysectomized *dog 7*. Preloban administration was continued for 5 more days (not shown in figure 2) on *dog 1*, to see whether a longer period would raise cardiac output and further increase oxygen consumption, but the dog was found dead on the morning of the 13th day, before an additional run was made. *Dog 4*, after both hypophysectomy and thyroidectomy, showed no change in cardiac output or oxygen consumption after 6 days of daily 225-unit preloban administration.

The findings on *dog 3* are complicated by the development of anemia. At her

second preoperative and her first postoperative (8th day) experiments there was no anemia, the arterial blood containing 20.1, 20.0 and 19.5 cc. of oxygen per 100 cc. At her second, third (without anesthesia), fourth and fifth postoperative experiments her arterial blood contained 16.9, 17.2, 14.0 and 12.8 cc. of oxygen per 100 cc. She was then put on 0.2 grams ferrous sulfate daily, continued for 30 days. The two experiments after beginning iron therapy showed falls in cardiac output, the last one showing the expected post-hypophysectomy value of

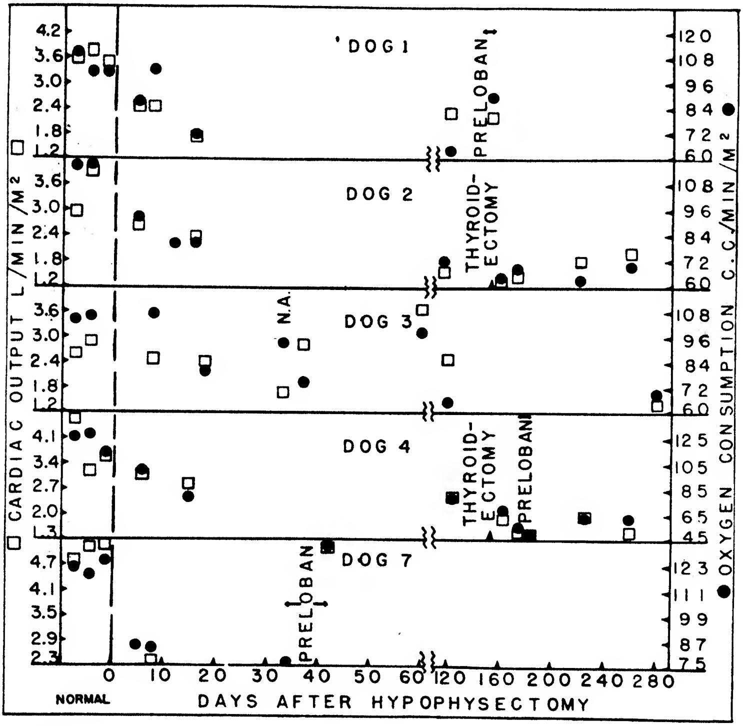

FIG. 2. Effects of hypophysectomy, of thyroidectomy and of preloban administration on cardiac output and oxygen consumption. The experiment on *dog 3* labelled N. A. was without anesthesia.

about 50% of normal. The interpretation of these findings, however, is by no means clear. The first experiment after iron therapy (6th postoperative experiment) gave a fall in cardiac output with a rise in arterial blood oxygen to 14.9 cc. per 100 cc.; but the last experiment of the series, with a still further fall in cardiac output, showed only 12.5 cc. of oxygen per 100 cc. arterial blood. Others have noted a rise in cardiac output in anemia (3, 4, 5); while the findings are not completely uniform, it appears that when the oxygen capacity of the blood falls to between 50 and 60% of normal, an increase in cardiac output is usually seen in dog and human subjects, the increase becoming greater with increasing severity

of anemia. The oxygen capacity of *dog 3* was at no time less than 60% of normal; if the relatively high cardiac outputs of the 4th and 5th postoperative experiments are due to anemia, it is difficult to explain the low output of the last experiment when (in spite of iron therapy) the anemia was equally severe. One might assume that the high value of 3.67 for the 5th postoperative experiment is in error; the other findings then agree fairly with those of the other dogs and the anemia can be considered as not playing a part; further speculation does not seem profitable. Anemia is not a typical finding after hypophysectomy; in no other case was there a post-hypophysectomy fall in arterial oxygen content, all samples being taken while the dogs were breathing pure oxygen.

Neural lobe denervation. Cardiac output and oxygen consumption were determined in 2 diabetes insipidus dogs (denervation of neural hypophysis). No preoperative determinations were made on these dogs; in our hands the Fick method in normal dogs has given cardiac outputs of 2 to 5 L/min/M^2, with an average of 3.8 L/min/M^2, while oxygen consumption is from 100 to 145, with an average of 125 cc/min/M^2. Diabetes insipidus *dog 27* had cardiac output and oxygen consumption values of 1.84 and 72 on the 44th postoperative day, and 3.0 and 126 on the 161st postoperative day; diabetes insipidus *dog 33* had corresponding values of 1.91 and 94 on the 32nd and 2.7 and 95 on the 125th postoperative day. The initial low values are ascribed to reversible operative damage to the anterior lobe; the late values are normal or close to low normal limits. There is no evidence that loss of neural hypophysis produces any great lasting change in cardiac output or oxygen consumption.

Discussion. The percentage falls in cardiac output following hypophysectomy and the percentage falls in cardiac output followed by return to normal following denervation of neural hypophysis are comparable with the changes in renal blood flow produced by the same procedures; the percentage falls in cardiac output following thyroidectomy are about twice as great as those in renal blood flow. In most cases a given procedure has a greater percentage effect on cardiac output than on oxygen consumption. Odaira (9) observed falls in cardiac output and oxygen consumption following thyroidectomy in rabbits comparable with ours in dogs; he also observed increases on thyroid feeding. Blalock and Harrison (10) found falls of 16 to 55% in cardiac output in dogs following thyroidectomy, with somewhat smaller falls in oxygen consumption. Most of their operations were subtotal thyroidectomies; following these there were signs of hyperthyroidism with increased cardiac output and oxygen consumption, the values falling on total thyroidectomy. None of their total thyroidectomy dogs survived longer than 3 weeks; these were not parathyroid deaths but were 'apparently from inanition'. They also consistently found progressive anemia after either total or subtotal thyroidectomy. We have not seen such fatalities, our total thyroidectomy dogs surviving for months and eventually being sacrificed. Nor have we seen anemia after thyroidectomy; thus, *dog 8* before operation had 18.0, 17.2 and 17.2 cc. oxygen per 100 cc. arterial blood and 17.4, 16.5, 17.8, 17.4 and 17.1 on the 20th, 51st, 91st, 147th and 161st post-thyroidectomy days, while *dog 10* preoperatively showed 15.8, 14.1 and 14.8, with 15.9, 16.6, 16.6 and 15.0 on the 8th, 15th, 21st and 80th postoperative days.

The questions arise as to how loss of anterior lobe acts to reduce cardiac output and how anterior lobe administration acts to increase it. Since loss of thyroid decreases and thyroid administration increases cardiac output, it might be supposed that the anterior lobe effects are due solely to loss or excess of the thyrotrophic hormone. While this is no doubt a factor it cannot be the only one, since preloban administration to thyroidectomized dogs (fig. 1) significantly raised oxygen consumption in all 3 instances and raised cardiac output in 2 of 3 trials. There is no reason to believe that the rises seen on anterior lobe administration are due to its adrenocorticotrophic action; the basal rate of oxygen consumption in Cushing's disease is low or normal, not increased (11, 12, 13), and the renal blood flow in Cushing's cases has been found normal (14). One may postulate that the anterior lobe, in addition to its hormones trophic to other endocrine glands, produces a substance which directly influences the organism's metabolism in such a way as to bring about increased oxygen consumption, cardiac output and renal blood flow; this postulated substance also increases the rate of renal tubular transport of diodrast or of p-aminohippurate (1).

Our data suggest that after loss of anterior lobe has existed for a long time, conditions are not restored to normal by a short period of replacement therapy, while such therapy at a shorter interval after hypophysectomy is effective. Thus, 8 days of preloban administration begun on the 37th post-hypophysectomy day on *dog 7* produced a striking increase, while 7 days of such administration begun on the 147th day on *dog 1* was much less effective (fig. 2). Similarly, preloban is more effective 70 days after thyroidectomy in *dog 10* than after 140 days on *dog 8* (fig. 1). While such findings do not permit final conclusions, they suggest that slowly progressive changes occur which make it increasingly difficult to obtain a response.

SUMMARY

1. Preloban (anterior lobe) administration produces increases in cardiac output and in oxygen consumption in the normal dog comparable with the previously reported increase in renal blood flow.

2. Thyroidectomy significantly reduces cardiac output and oxygen consumption. Preloban administration to the thyroidectomized dog usually somewhat increases both cardiac output and oxygen consumption, indicating that its action is not entirely through its content of thyrotrophic hormone; the effect is not as great as in the normal dog. The formation by the anterior lobe of a substance which directly influences metabolic processes is postulated.

3. Hypophysectomy produces a marked, prompt and permanent fall in cardiac output and oxygen consumption comparable with that previously reported in renal blood flow; subsequent thyroidectomy produces a slight further fall.

4. Preloban administration to the hypophysectomized dog raises cardiac output and oxygen consumption, usually to normal values; it apparently becomes less effective with increasing time after either thyroidectomy or hypophysectomy.

5. Cardiac output and oxygen consumption were found within normal limits in dogs with chronic denervation of neural hypophysis.

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AN EVALUATION OF A METHOD INVOLVING CARBON DIOXIDE EQUILIBRATION FOR DETERMINING CARDIAC OUTPUT[1]

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Received for publication August 15, 1947

The total pulmonary blood flow of the normal individual may be determined by applying the Fick principle (1) to blood oxygen and carbon dioxide values obtained from simultaneous direct sampling of the pulmonary artery and a peripheral artery and from determinations of the respiratory gas exchange (2). In many cases of congenital heart disease, however, the total pulmonary blood flow (pulmonary capillary flow) may exceed the blood flow through the pulmonary artery because of the presence of large collateral circulation to the lungs or the persistence of a patent ductus arteriosus. Consequently, in studies reported in earlier papers of this series (3–6) an indirect method was used for the determination of pulmonary capillary blood flow. Pulmonary artery blood flow, on the other hand, was determined by direct catheterization of the right ventricle or pulmonary artery.[2]

In the normal individual, collateral circulation to the lung is insignificant (7). In the normals, values for cardiac output using a method based on the indirect Fick principle should equal those derived from an application of the direct Fick principle. From the results thus obtained, and from the values for pulmonary artery flow, the collateral circulation to the lung can be calculated (3).

It is the purpose of the present communication to test the accuracy of the indirect method by comparing its results with those obtained from the application of the direct Fick principle. The results were obtained from a group of 14 subjects having no known intracardiac shunts. All subjects were volunteers from the patient population of the hospital.

METHODS. Many indirect methods of determining the cardiac output by means of the Fick principle have been based on the rate of diffusion of inert gases

[1] This study was supported by a grant from the Commonwealth Fund and the Carolyn Strauss Fund.

[2] According to the Fick principle, formulae for calculation of cardiac output in the normal individual by the direct method and pulmonary capillary flow by the indirect method are

1. $$\text{(Pulmonary artery blood flow (cardiac output) ml/min.)} = \frac{\text{Oxygen intake in ml./min.}}{O_2 \text{ content of femoral arterial blood} - O_2 \text{ content of right auricular blood}} \times 100$$

2. $$\text{(Pulmonary capillary blood flow in ml/min.)} = \frac{\text{Carbon dioxide output in ml./min.}}{CO_2 \text{ content of blood reaching alveoli} - CO_2 \text{ content of pulmonary vein blood}} \times 100$$

The flows are expressed as liters/minute/M^2 body surface area.

from the pulmonary alveoli into the blood. Various workers have used nitrogen (8), nitrous oxide (9), ethyliodide (10) and acetylene (11). Others have employed the Fick principle by determining indirectly the gas contents of pulmonary capillary and pulmonary vein blood and the exchange of one of the respiratory gases. Most of these studies have used carbon dioxide, chiefly because of its greater diffusion rate. The method to be presented falls in this group.

The difficulties and theoretical objections of the indirect method involving carbon dioxide equilibration have been thoroughly discussed in the reviews of Richards and Strauss (12), Haldane and Priestley (13), Marshall (14), Grollman (15), Morrissey (16) and Hamilton (17).

Determination of the carbon dioxide content of pulmonary capillary blood. Löwy and von Shrötter (18) attempted to equilibrate the alveolar air in a lobe of the lungs with pulmonary capillary blood by blocking off the bronchus to that lobe with a balloon-encircled bronchial catheter. They waited until the carbon dioxide tensions in the test portion of the lung and the pulmonary capillary blood were in equilibrium. Such a state was indicated when repeated sample values formed a plateau. This method was cumbersome and in some cases hazardous enough to discourage its frequent application. This complicated method was replaced by Plesch (19), who advanced the idea that the desired equilibrium would be reached if, after rebreathing expired air from a bag several times, a constant value for these gases was found in successive samples from the bag.

Early experience in this laboratory demonstrated that any equilibration method for determining the carbon dioxide content of pulmonary capillary blood in congenital heart disease had to have several special qualities. First, the execution of the test had to be simple enough to be performed by very young subjects. Second, equilibration had to be completed before the recirculation of carbon dioxide began (17). The possibility of recirculation is considerably greater than normal in the presence of septal defects with intracardiac shunts. Third, it had to meet the criteria for accurate equilibration which involved: *a*) oxygenating the pulmonary capillary blood so that constant 'virtual' carbon dioxide values could be determined from an oxygenated carbon dioxide dissociation curve; *b*) holding the inspired mixture in the lungs for a sufficient time to produce intrapulmonary mixture of gases; *c*) minimizing changes in cardiac output incident to the procedure itself. Although it is recognized that breath-holding itself will alter cardiac output (17), the effects of such a change would not be reflected appreciably in changes in the carbon dioxide tension of pulmonary capillary blood until a complete circulatory cycle was approached. Hence the data obtained in the allotted time would still represent basal conditions.

After getting unsatisfactory results from rebreathing methods of attaining equilibration, a technique was devised which modified the methods of Christiansen, Douglas and Haldane (20), Henderson and Prince (21), Burwell and Robinson (22), Field and co-workers (23) and Richards (24). As far as possible, the method adopted met the requirements for general cardiac output studies as well as the special criteria for work in congenital heart disease.

Three anesthesia bags, each provided with a three-way tap, were filled from commercial tanks with specially prepared oxygen-carbon dioxide mixtures of approximately 4, 5 and 6% carbon dioxide, respectively. The carbon dioxide percentages used covered the range expected in the pulmonary capillary blood. The small difference in the percentages was necessary because it was found that rapid equilibration was impossible if the carbon dioxide tension in any bag differed more than 2% from that of the pulmonary capillary blood (3). The volume of each bag was adjusted to about 60% of the patient's vital capacity. A rubber mouth piece attached to one arm of the three-way tap was placed in the patient's mouth. After allowing the patient to breathe with the tap turned to room air until he was adjusted to the apparatus, he was instructed to take a full inspiration. At the beginning of inspiration the tap was turned from room air to the gas bag. After holding the mixture in his lungs for a period not exceeding 8 seconds, but greater than 5 seconds, the patient made a deep complete expiration into the bag, and the tap was turned to room air. Following an interval of 5 minutes, the procedure was repeated with the same bag. The content of the bag was then analyzed for carbon dioxide. Each bag was equilibrated in turn and the results analyzed. In most cases the contents of the bags agreed within 0.1%. In the event that agreement was not reached, a rough extrapolation of the direction of change usually indicated the mixture which had not equilibrated, and one or two further re-equilibrations brought it into accord. Upon reaching a satisfactory agreement, an average of the carbon dioxide contents of the bags was determined, and the results, in terms of mm. Hg pressure, applied to an oxygenated carbon dioxide dissociation curve for conversion into blood carbon dioxide contents.

Determination of the carbon dioxide content of pulmonary vein blood. In the presence of intracardiac shunts, peripheral arterial blood carbon dioxide content cannot be accepted as representative of the carbon dioxide content of the pulmonary vein blood (25). Hence the carbon dioxide content of pulmonary vein blood must be estimated by indirect means. It has been shown by Comroe (26) among others that alveolar gases are in equilibrium with the blood gases of the arterial blood in normal individuals; consequently, it is possible to determine the carbon dioxide content of pulmonary vein blood from the partial pressure of carbon dioxide in alveolar air.

Most methods of determining the alveolar gas tensions follow or are modifications of the method of Haldane and Priestley (27). Having shown that the alveolar gas tensions and pulmonary vein blood gas tensions varied somewhat with the respiratory cycle, these authors attempted to approach a mean value for the alveolar gas tensions. They trapped gas from the end of a deep, rapid expiration in a pre-evacuated mercury sampling tube. The samples were taken at the end of a normal inspiration and at the end of a normal expiration. The average of the two values (inspiratory and expiratory alveolar air, respectively) was accepted as representative of the mean alveolar gas tension.

Since there has been a great deal of difficulty in obtaining expiratory alveolar air samples from children, only inspiratory alveolar air samples have been collected. For reason of standardization this method of collecting alveolar air

has been employed in the case studies presented here. Usually the average of several closely agreeing alveolar air samples was used.

Some of the patients in the younger age group were not able to cooperate in this test even after repeated attempts; hence the Smith and Heinbecker valve for sampling alveolar air (28) has been used in a few of the very young children having congenital heart disease. Results obtained in this way from older patients agreed closely with those from the method of Haldane and Priestley (27).

The determination of the total carbon dioxide output. In order to determine the total amount of carbon dioxide produced, the patient's expired air was collected under basal conditions for a period of 1 to 2 minutes and analyzed for carbon dioxide and oxygen.

The construction of an oxygenated carbon dioxide dissociation curve. The results of the equilibration and alveolar air techniques were expressed as carbon dioxide tension in mm. of Hg. In order to calculate the volume flows, the gas tensions had to be converted into the volumes of gas per 100 ml. of blood. This was done by reference to an oxygenated carbon dioxide dissociation curve.

It has long been recognized that a separate curve must be determined for each individual because of variations in hemoglobin content and pH of the blood (29). The construction of a carbon dioxide dissociation curve was made easier when Peters and co-workers demonstrated that the carbon dioxide dissociation curve plotted on logarithmic graph paper was represented by a straight line. The slope of this line could be calculated from the oxygen capacity of the blood (30, 31). Hence it was possible to construct a curve from two observed points, or, according to Peters, from one point through which a line of calculated slope could be drawn.

In the method reported here, pulmonary capillary blood was equilibrated with a gas mixture containng 94 to 96% oxygen; therefore, it was necessary to construct dissociation curves from fully oxygenated blood. This was done by equilibrating 5 ml. samples of blood with two oxygen-carbon dioxide mixtures containing 4 and 7% carbon dioxide, respectively The equilibration was carried out in glass tonometers rotated in a water bath at 37°C. for 20 minutes. Immediately following the equilibration the carbon dioxide in the gas mixture and in the blood was determined. By plotting these data on logarithmic graph paper, two points were obtained. In most cases it was found that the slope of a line drawn through the two observed points coincided closely with the slope calculated by the Peters formula.

The method just described for obtaining a fully oxygenated carbon dioxide dissociation curve introduced a slight error, since the blood in the pulmonary vein is slightly below 100% in oxygen saturation. Therefore, the true dissociation curve for the alveolar air sample values would be shifted somewhat to the left of the constructed curve. The error was not considered sufficient to warrant applying a correction factor.

The direct Fick procedure. Right heart catheterization was used in the determination of cardiac output by means of the direct Fick principle (2). It is realized that this method is not without error (32). However, its results appear to be more reliable than those obtained with other procedures.

In the cases reported in this paper it has been impossible to obtain simultaneous determinations for the indirect and direct method. However, the two methods have been applied in immediate sequence. Usually the indirect was followed at once by the catheterization procedure. The usual sequence of events for the indirect test was first, a determination of the carbon dioxide produced; second, the collection of several alveolar air samples; and third, the equilibration for estimating carbon dioxide content of pulmonary capillary blood. The calculations were then made according to the formulae presented.

Technical methods. All gas analyses were carried out in the Haldane gas analysis apparatus. The results of these analyses, expressed as per cent, were multiplied by the barometric pressure from which 47 mm. of Hg, representing water vapor pressure, had been subtracted. The value thus obtained was the gas tension in mm. of Hg. All gas volumes were expressed in terms of dry gas at 760 mm. mercury and at 0°C.

The blood-gas contents were determined in the Van Slyke manometric apparatus and expressed as volumes of gas per 100 ml. of blood.

Blood samples were drawn under oil in syringes containing 2 mgm. of sodium fluoride and 15 mgm. of potassium oxalate per 10 ml. of blood.

RESULTS AND DISCUSSION. In comparing the values obtained from the various procedures of the indirect method with their counterparts in the direct method, considerable deviation was found. The deviation of the pulmonary capillary blood (indirect method) carbon dioxide content from that of the pulmonary artery (direct method) had a range of −8.1 vol. % to +4.4 vol. %. The deviation of pulmonary vein blood carbon dioxide content determined by the alveolar air sample (indirect method) from that of the peripheral arterial blood (direct method) had a range of −3.8 vol. % to + 5.0 vol. %. The disagreement between the carbon dioxide figures determined by direct analyses and by calculation might be interpreted as proof of the contention that the carbon dioxide content of the inspired mixture is diluted with residual air. Thus the resulting concentration of carbon dioxide in the lung air does not rise sufficiently to effect a rapid equilibration with pulmonary capillary blood (17). It is conceivable, however, that the large differences observed may be caused by other factors. It has been shown by Cournand that values for cardiac ouput as determined from the carbon dioxide contents of catheter blood do not check with those obtained from oxygen figures (2). This suggests that the individual carbon dioxide values determined directly cannot be used as absolute reference points. Furthermore, the two tests were performed in sequence rather than simultaneously. It is conceivable that the carbon dioxide values in pulmonary blood change during the period elapsing between the two procedures without a corresponding alteration in the cardiac output. These considerations indicate that the difference between the carbon dioxide values obtained by the two methods is not necessarily the result of insufficient equilibration of the inspired carbon dioxide with pulmonary capillary blood. Despite these considerations it cannot be denied that the latter factor may play some part in the results obtained.

The results obtained for the cardiac output by the two methods, on the other hand, show better agreement (table 1). The two methods were related by a

correlation coefficient having a value of 0.68 with a standard error of 0.28. In 11 of the 14 cases the results of the indirect method fell within 30% of the standard direct method. In three cases (Nos. 1, 4 and 14) in which the deviation was greater than 30%, respiratory quotients of 1.17, 1.2 and 1.17, respectively, were obtained from the metabolism tests (table 1). This clearly represents overventilation on the part of the subject, and these cases should be considered unsuitable for an indirect determination of cardiac output.

TABLE 1.

CASE NO.	SURFACE AREA M^2	INDIRECT FICK				DIRECT FICK						%[1] Difference
		Incoming CO_2	Outgoing CO_2	CO_2 Produced	Pul. Cap. Flow	Pul. Art. O_2	Pul. Art. CO_2	Fem. Art. O_2	Fem. Art. CO_2	O_2 Intake	Cardiac Output	
		vol.%	vol.%	cc/min.	L/min/M^2	vol.%	vol.%	vol.%	vol.%	cc/min.	L/min/M^2	
1	1.7	38.2	35.7	179	4.2	17.7	37.2	22.0	36.0	210	2.9	45.0
2	1.62	63.0	60.0	127	2.6	13.8	60.0	17.8	60.0	188	2.9	10.0
3	1.46	39.1	35.0	126	2.1	14.1	41.3	19.6		172	2.1	0
4	1.46	53.1	50.0	160	3.5	13.6	57.8	19.1	45.0	175	2.2	59.0
5	1.6	36.0	32.3	170	2.9	R.V.[2] 15.0	36.2	19.1	34.0	214	3.3	12.0
6	1.57	48.7	46.3	183	4.8	14.2	51.2	18.1	46.3	226	3.7	30.0
7	1.55	51.1	46.5	146	2.1	R.A.[3] 15.9	49.9	20.9	46.5	184	2.4	12.5
8	1.49	52.9	49.8	176	3.8	R. V. 6.6	48.5	10.4	48.2	188	3.4	12.0
9	1.45	39.0	36.5	132	3.6	R. V. 12.5	39.3	16.4	40.3	204	3.6	0
10	1.43	44.5	40.5	120	2.1	12.5	44.0	15.7	41.6	109	2.4	12.5
11	1.58	38.8	36.1	138	3.2	13.5	40.9	16.2	38.5	115	2.7	18.5
12	1.57	40.8	37.4	155	2.9	R. V. 9.5	48.9	14.1	45.2	163	2.3	26.0
13	1.49	42.0	37.9	151	2.5	R. A. 9.9	41.7	13.2	37.9	143	2.9	14.0
14	1.40	45.6	42.7	169	4.1	15.1	46.4	19.1	42.7	144	2.6	58.0

[1] $\% \text{ difference} = \dfrac{\text{Direct} - \text{Indirect Flow}}{\text{Direct Flow}} \times 100$

[2] R. V. = Right Ventricle

[3] R. A. = Right Auricle

It is realized that the small number of experiments reported does not permit an accurate statistical analysis of the probable relationship between the methods in an unlimited population. A further difficulty lies in the likelihood that a group of volunteers would have a greater degree of cooperativeness than the general population, hence would constitute a 'selected' sample. However, it is improbable that the agreement between the two methods could be entirely the result of cooperation, and even if it were, the method would be of value in those individuals who are able to cooperate.

In view of the results of these experiments, differences between the direct Fick determination of pulmonary artery flow and the determination of pulmonary capillary flow of less than 30% cannot be considered evidence of collateral

circulation in congenital heart disease. Conversely, differences over 30% should suggest the presence of collateral circulation.

SUMMARY

Results of studies of 14 subjects having no known intracardiac shunts were reported comparing the indirect determination of pulmonary capillary flow with the direct right heart catheterization method.

A correlation coefficient of 0.68 was found relating the two methods.

Errors of the method and its application to studies of congenital heart disease were discussed.

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RELATION OF THE SALIVARY FLOW TO THE THIRST PRODUCED IN MAN BY INTRAVENOUS INJECTION OF HYPERTONIC SALT SOLUTION[1]

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Received for publication September 23, 1947

Subjectively the sensation of thirst is attributed to dryness of the mouth and throat. Cannon (1) demonstrated that there was a close correlation between the sensation of thirst and a reduction in the salivary flow. In seeking an explanation for this reduction in salivary flow and also in relating thirst to fluid changes in the body Gregersen demonstrated concurrent reductions in both plasma volume and salivary flow during periods of thirst produced by water deprivation, sweating, severe diuresis and hemorrhage (2, 3, 6). This evidence coupled with Gesell's observations (4, 5) suggested that a decreased blood supply to the salivary glands could account for the diminution in salivary secretion.

Leschke (7) in 1918, and more recently other investigators (8, 9, 10), have demonstrated that thirst can be induced by the intravenous injection of hypertonic salt solution. This produces an increase in the plasma volume (10), and therefore represents a situation in which the above explanation would not seem to apply. However, other mechanisms may be operating. Furthermore, we could find no data on the effect of intravenous injection of hypertonic salt solution on salivary flow. Hence the following study was undertaken to make such measurements in man and to determine the relation, if any, between changes in salivary flow and the sensation of thirst.

Procedure. All subjects were adult males, ranging in age from 30 to 60 years, who were receiving salt injections for the treatment of peripheral vascular disease (thrombo-angitis obliterans or arteriosclerosis). On the day of the experiment an early breakfast was restricted to coffee and fruit juice. The patient was allowed water ad libitum from the time he arose in the morning until he was seen in the out-patient clinic at 10 A. M. After a rest period of approximately 30 minutes, each subject received from 250 to 300 cc. of 5% NaCl injected into the antecubital vein over a period of 15 to 20 minutes. After a variable period of time (10 to 60 minutes), the subjects were allowed water. A record was kept of the water intake until 6 P. M. No food was taken until approximately 2 hours after completion of the injection. At this time all tests had been carried out and the subjects were allowed to return to their daily routine.

[1] The authors are indebted to Dr. Beverly C. Smith of the Department of Surgery, College of Physicians and Surgeons, Columbia University, for his cooperation in these studies, which were carried out in the Peripheral Vascular Clinic, Vanderbilt Clinic, Columbia University, New York City.

[2] Present address: University of Colorado School of Medicine, Denver, Colo.

Salivary flow was measured by collecting the saliva during a 5-minute period of breathing through the mouth (3). At least 2 measurements of salivary flow were made prior to the salt injection, and the determinations were repeated immediately after completion of the injection. In many experiments salivary flow was also measured at regular intervals after the ingestion of water.

In 9 experiments venous blood samples were taken before the injection, immediately afterward, 30 minutes later and in some instances also at 15 and 60 minutes after the injection. Following the administration of salt, all blood samples were drawn from the opposite arm and care was taken to avoid stasis. No water was permitted until the last blood sample was obtained. The serum protein concentration was determined by the refractometric method of Neuhausen and Rioch (12), and the serum chloride concentration by the absorption

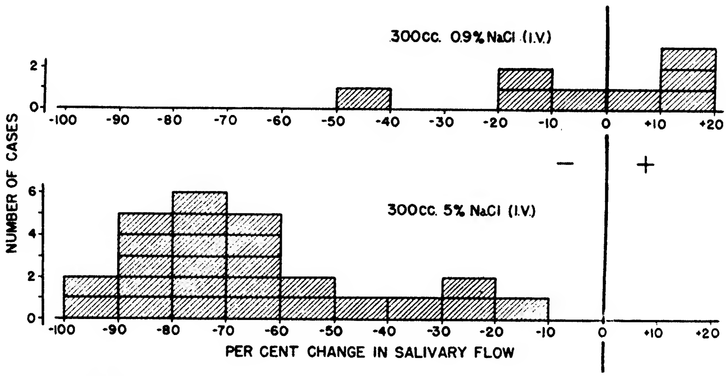

FIG. 1. Results of 25 experiments on 16 human subjects (lower graph) showing the per cent decrease in salivary flow that occurred immediately after intravenous injection of 300 cc. of 5% NaCl. The salivary flow was essentially unchanged in 8 experiments on 5 subjects receiving 300 cc. of 0.9% NaCl (upper graph).

indicator method of Saifer and Kornblum (13). In 5 experiments measurements were also made of the hematocrit value, using the Wintrobe tube, and of the serum sodium concentration, using the zinc uranylacetate method of Butler and Tuthill (14).

RESULTS. Twenty-five experiments were performed on 16 subjects. It can be seen from figure 1 (lower graph) that in every instance the salivary flow was reduced immediately after the injection. In 80% of the experiments the flow was reduced by more than 50% of the control value. The drinking of water relieved the thirst and restored the salivary flow to normal levels (fig. 2). If the subjects were not allowed to drink until 30 to 60 minutes after completion of the injection, the reduction in salivary flow persisted until drinking was permitted.

The subjects complained of dryness of the mouth after the injection of only 100 to 150 cc. of the 5% salt solution. As the injection was continued the thirst became more intense. The patients stated that the saliva became thick, and

sticky and had a peculiar taste, and that rinsing the mouth with water gave little relief. In describing the taste sensation noted after the injection, most of the men used the word 'peculiar' and were not completely satisfied with such terms as 'metallic' and 'salty'. Whenever permitted the subjects would drink 200 to 800 cc. of water immediately. The extent of the drinking appeared to be limited by the severity of 'thirst', 'full feeling in the stomach' and 'coldness of the water'. The subject might take additional water in 10 to 15 minutes and then there would be no further drinking until 1½ to 2 hours later. There was a rough correlation between the percent reduction in salivary flow after salt injection and the amount of water a subject would drink.

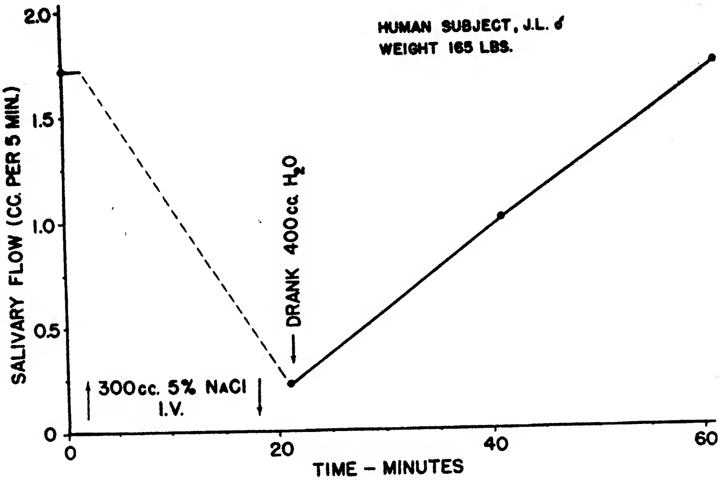

Fig. 2. Reduction of salivary flow after intravenous injection of 300 cc. of 5% NaCl and the return to control values after the drinking of water, in subject J. L.

The salt injection caused a rise in the serum chloride concentration. The average increase was 9.8 mEq. immediately afterward and 7.3 mEq. 30 minutes later (table 1). The serum protein concentration and hematocrit values dropped as a result of the injection. The average decrease in the serum protein concentration was 1.09 gram % immediately after the injection and 0.74 gram % 30 minutes later (table 1). The average decrease in the hematocrit value (percent red cell volume) was 5.0 and 2.7, respectively, for the same periods. Calculations of the increase in plasma volume based on the changes in hematocrit value and concentration of plasma protein and of the increase in extracellular volume based on the changes in concentration of serum sodium and chloride (16) indicated that there was a 5 to 10% increase in both plasma volume and extracellular fluid volume as a result of the salt injection. These calculations agreed with the volume changes observed by Dr. Ferrebee in these same patients using the blue dye T-1824 for the determination of plasma volume and NaSCN for the determination of extracellular volume (15).

Later in this study the opportunity of obtaining control data was realized when the treatment was changed so that 5 of the subjects received 300 cc. of 0.9% NaCl and one-half grain of papaverine instead of 5% NaCl. The results in this group (8 experiments) differed markedly from those of the previous experiments (figure 1, upper graph). In only one instance was there a distinct reduction in salivary flow (40%). In the other 7 experiments there was no significant change. These subjects did not know that their treatment had been altered, and they could not understand why they did not feel thirsty after the injection.

TABLE 1. *Changes in serum chloride concentration and serum protein concentration observed following the intravenous injection of 5% salt solution in 9 men. In the case of H, 600 cc. of water was given by mouth 25 minutes before starting the salt injection*

SUBJECT	DATE	NO. CC. SALT INJECTED	SERUM Cl (M.EQ. PER LITER) Control	Immediately after NaCl	30 min. after NaCl	MAXIMUM RISE IN Cl	SERUM PROTEIN (GRAM PER CENT) Control	Immediately after NaCl	30 min. after NaCl	MAXIMUM RISE IN PROTEIN
Re........	2/29/39	300	96.0	104.0		8.0	7.88	6.60		1.22
Le........	3/14/39	300	98.0	105.5		7.5	6.85	5.83		1.03
M........	4/ 4/39	300	97.0	111.0	105.0	14.0	6.86	5.67	6.24	1.49
G........	4/11/39	195	94.0	102.0	101.0	8.0	5.31	4.63	4.84	0.68
Li........	10/17/39	300	98.5	108.2	107.0	9.7	8.53	6.68	7.14	1.85
A........	10/ 4/39	300	100.0	110.0	106.0	10.0	5.66	5.05	5.15	0.61
D........	10/ 7/39	300	100.8	110.5	105.2	9.7	7.14	6.43	6.58	0.71
Ro........	10/24/39	300	97.5	108.2	108.3	10.7	7.83	6.60	7.47	1.23
B........	11/24/39	300	100.2	110.4	107.5	10.2	7.21	6.34	6.75	0.87
						9.8				1.09
H........	4/18/39	250	98.0 97.0[1]	110.0		12.0	7.83 7.63[1]	6.70		1.13

[1] Values obtained 25 minutes after ingestion of water and immediately preceding salt injection.

As a further control, measurements of salivary flow were made approximately 5 times a day over a period of 2 weeks on a group of 5 laboratory workers. The salivary flow from day to day was remarkably constant and indicated that the results shown in figure 1 could not be attributed to normal daily fluctuations in salivary flow.

In a previous study in dogs (10) we had shown that if water was given by gastric fistula 15 to 30 minutes before the intravenous injection of hypertonic salt solution (20%), the usual drinking response which followed such injection was either minimal or absent. We wanted to see if similar results could be demonstrated in man. In 8 experiments 5 subjects ingested 400 to 600 cc. of water 20 to 30 minutes before salt injection. All testified that this procedure relieved the intense thirst which usually followed the injection of 5% salt. In several instances they stated that they were not thirsty at all and drank no water after the

injection. The results of the experiments on 4 of these subjects are shown in table 2. It will be noted that previous ingestion of water effectively inhibits the marked drop in salivary flow that is always observed after the intravenous injection of hypertonic salt solution. Here again changes in salivary flow appear to be indicative of the degree of thirst experienced. Blood studies were made in one of the tests. It was found that the administration of water prior to the salt injection in no way altered the nature or the degree of the rise in chloride concentration or the fall in the protein concentration (table 1, subject H). This is in agreement with the results from similar tests in dogs (10).

DISCUSSION. The above results provoke an interesting speculation. To be sure they appear to be in accord with Cannon's hypothesis that thirst arises from a deficient salivary flow and a sense of dryness in the buccal region. Indeed, the subjects themselves testified that the thirst sensation was localized in the oral cavity, stating that this region felt dry and that the saliva was thick and sticky. What stands out as a mystery is how the reduction in salivary flow occurs, for in this instance it cannot be ascribed to a diminished blood volume. On the contrary the salt injection increases the blood volume 5–10%.

TABLE 2. *Experiments on 4 subjects showing that the ingestion of 600 cc. of water 20 to 30 minutes before injection prevents the intense thirst as well as large reduction in salivary flow which follows the intravenous administration of 300 cc. of 5% NaCl*

SUBJECT	INTRAVENOUS INJECTION OF 300 CC. OF 5 % NaCl			600 CC. WATER GIVEN 20 TO 30 MINUTES PRIOR TO INJECTION OF 5 % NaCl		
	Date	reduction salivary flow	Thirst	Date	change in salivary flow	Thirst
		%			%	
L.	1/17/40	92	++++	2/ 4/40	−13	+
	2/10/40	88	++++			
	3/14/40	70	++++			
H.	1/17/40	87	++++	1/24/40	−10	0
	1/10/40	75	++++	2/ 4/40	−35	+
				3/29/40	−7	0
				4/18/40	−14	0
C.			++++	3/21/40	+30	0
E.			++++	7/12/39	−20	+

The absence of thirst and the maintenance of normal salivary flow when water is ingested prior to the salt injection is also rather puzzling, because this procedure does not materially alter the response to salt injection so far as the rise in serum chloride or drop in plasma protein and hematocrit values are concerned. This would suggest that further study of the mechanism of changes in salivary flow might reconcile Cannon's theory of the local origin of thirst with the concept of thirst as a sensation of diffuse origin (17). Proponents of the latter concept have suggested several factors as playing a role in the initiation of thirst, namely, increased serum osmotic pressure (18), increased serum sodium (7, 19) and decreased water content of tissue cells (8, 17). From the blood studies it would appear that all of these changes were present following the salt injection, but

there was also an associated reduction in salivary flow. Furthermore, when water was given prior to the salt injection, the salivary flow was correlated with the sensation of thirst but the blood changes measured in this study did not appear to be so correlated.

SUMMARY

In man (25 experiments) the thirst produced by the intravenous injection of 300 cc. of 5% sodium chloride was found to be associated with a reduction in salivary flow. This is consistent with the 'dry mouth' theory of thirst. The changes in serum concentration of sodium, chloride and protein following the injection indicated an increase of 5 to 10% in plasma and extracellular volumes.

Ingestion of water (400 to 600 cc.) 20 to 30 minutes prior to the salt injection alleviated the severe thirst usually experienced and prevented the reduction in salivary flow. Nevertheless the rise in serum chloride concentration and the drop in serum protein concentration were of the same degree as in the other experiments. This suggests that thirst and salivary flow are sensitive to fluid changes which are not measured by such indices as changes in serum concentration of protein, chloride and sodium.

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DISPLACEMENT OF BLOOD FROM THE LUNGS BY PRESSURE BREATHING[1]

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Received for publication September 23, 1947

The work reported in this paper was part of a study of the effects of positive pressure breathing on the peripheral circulation. A general survey of this field has been recently published by Barach, Fenn, Ferris and Schmidt (1). Evidence of decreased blood flow through the fingers has been reported elsewhere (2). This paper gives further evidence that pressure breathing causes a peripheral vasoconstriction. Furthermore, by a combination of a teeter-board and plethysmographic method an approximate calculation is made of the volume of blood (500 cc.) displaced from the lungs during pressure breathing at 30 cm. H_2O.

METHOD. The teeter-board or balance board was made of plywood on a wooden frame, *B*, 7 feet long and 2½ feet wide and was supported by knife edges, *e*, at its mid point as shown in figure 1. The subject lay supine on the board on a narrow mattress and his position was so adjusted that the foot end of the board was slightly heavier than the head end. This excess weight at the foot was supported by a steel spring attached to the board by a wire and supported on a table above the board. The spring was a flat steel strap, *S*, 9 x ¾ x 16 inches, firmly supported at one end. Movements of the free end were magnified and recorded by a thread attachment to a heart lever, *L*, writing on a smoked drum. The foot of the teeter-board was attached by a wire close to the point of support of the spring which functioned therefore like an isometric lever. The sensitivity of this system was such that a deflection of 1 cm. on the record was produced by the addition of a 270-gram weight at the foot of the board 106 cm. from the knife edge or by a torque of 28.6 kgm. cm.

The teeter-board serves to measure the total change in torque due to the blood displaced, but does not tell precisely where the blood came from nor where it went to. To assist in the interpretation of the torque, therefore, one lower leg up to a point just below the knee was enclosed in a plethysmograph, *P*, of galvanized iron filled with air. Changes in volume of the plethysmograph were recorded by an Eberbach volume recorder, *V*, writing on a smoked drum. The sensitivity was such that a deflection of 1 cm. indicated a change in volume of 10.6 cc. The orifice of the plethysmograph was sealed airtight around the leg with plasticene and zinc ointment. This caused no interference with the venous return, and leaks could easily be detected by injecting air into the system.

[1] This work was performed under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of Rochester. It was reported in December 1943 to the Committee on Aviation Medicine as Report No. 249.

This raised the recorder to a new level which was maintained constant so long as the system was airtight.

Results. *Occlusion of the venous return from the legs.* For an initial test of the apparatus, blood pressure cuffs were wound around both legs just below

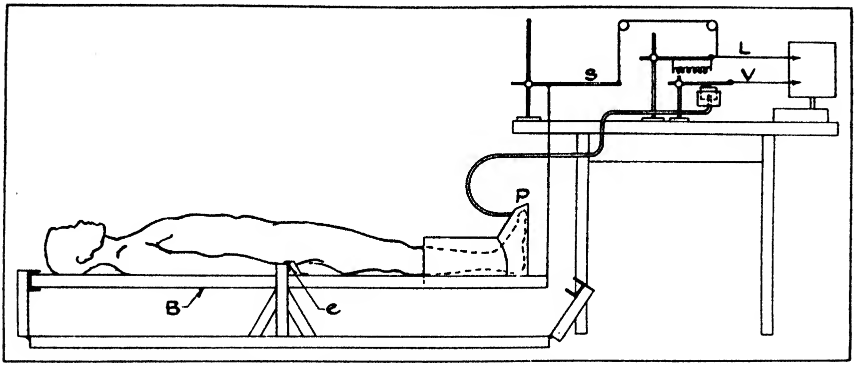

Fig. 1. Diagram of teeter-board and plethysmograph methods. Devices are provided for locking the teeter-board at both ends when not in use.

Table 1. *Effect of inflating cuff on leg*

SUBJECT	Δ VOL. IN BOOT (a)	Δ TORQUE (b)	RATIO LEGS/FOOT (c)	Δ BLOOD BELOW CUFF (d)	DISTANCE CALCULATED (e)	5TH I.S. TO KNEE (f)
	cc.	*kgm. cm.*		*grams*	*cm.*	*cm.*
Hegnauer	24.7	13.3	5.5[1]	143	93	81
Chadwick	40.6	18.6	5.0	213	87	84
Fenn	37.2	15.5	5.5	215	72	74
Rahn	39.0	17.5	4.6	188	93	90
Epstein	51.7	22.7	5.1	277	82	81
Average	38.6	17.5	5.1	207	85	82

[1] Assumed value.

Columns (a) and (b) contain the experimentally measured values resulting from the inflation of the cuff. Column (c) is the volume of both legs below the cuffs divided by a volume of the foot in the plethysmograph. The value for Hegnauer is assumed as probably nearly the same as for Fenn (measured for the others). Column (d) is the product of (a) × (c) × density of blood, 1.05. It represents the total amount of blood dammed up below the cuff if all the leg accumulates as much per liter as the foot. Column (e) is column (b) divided by column (d). Column (f) is the distance on each subject from the fifth intercostal space to the knee joint.

the groin. When these were inflated to 60 mm. Hg pressure so as to impede the venous return, the foot plethysmograph showed an increase in volume of 38.6 cc., and the change in torque on the teeter-board was 17.5 kgm. cm. These figures are averages from 5 subjects, the individual data being included in table 1. A typical record of cuff inflation is shown in figure 2. The volume of the

legs below the cuffs and the volume of the portion of the leg in the plethysmograph were determined by water displacement, and it was assumed that all the tissue in both legs below the cuff received as much blood per liter of tissue as the part in the plethysmograph. On the average then, it may be calculated that the increase in blood in the two legs below the cuffs was 207 grams (assuming a blood density of 1.05). In order to explain an increase in torque in the footward direction of 17.5 kgm. cm., 207 grams of blood must have moved an average distance of 17.5/207 or 85 cms. which is approximately the distance from the knee to the heart or lungs. The knee may be taken as the center of gravity of the legs below the cuffs, and the heart then represents the center of gravity of the origin of this volume of blood. The agreement shown in the last two columns in table 1 between this calculated distance and the measured distance from the fifth intercostal space to the knee is quite remarkable. The experiment indicates that the plethysmograph and the teeter-board agree reasonably well in their

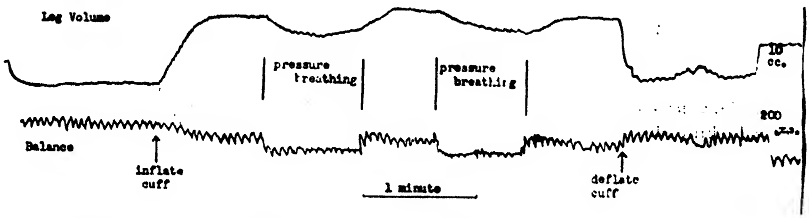

FIG. 2. EVIDENCE FOR VASOCONSTRICTION IN THE FOOT DURING PRESSURE BREATHING. When a cuff is inflated (*arrow*) around one leg the foot volume increases in the plethysmograph (*upper record*) and the foot-end of the teeter-board grows heavier (*down stroke in lower record*). With the cuff still inflated pressure breathing increases still further the weight of the foot-end of the board but the foot protected by the inflated cuff decreases in volume. At the right are calibration deflections obtained with 10 cc. and 200 grams, respectively.

readings and suggest further that the lungs act as a storage depot for blood from which supplies may be taken when any part of the normal blood volume is trapped in the periphery by venous occlusion.

Pressure breathing. In other experiments we have measured the effects of pressure breathing on the foot volume and the balance of the teeter-board. The positive pressure was applied through a pressure mask connected with a stream of air and a spring-loaded outflow valve. During the experiment the pressure was increased for a few minutes or until the circulatory response was complete, and then was released. In this procedure there seemed to be danger of an artifact because of the descent of the diaphragm during the pressure breathing. This increases the weight of the foot of the board and tends to obscure the change in weight due to the movement of blood. In order to take account of this error, we endeavored always to make readings when the diaphragm was in the same position. This was accomplished by placing two pneumographs on the subject, one around the thorax and one around the abdomen. These were attached to water manometers which were set to read zero at the end of a normal

inspiration. The manometers were placed in view of the subject. Before, during and after pressure breathing, the subject was asked to 'level off' by expiring or inspiring until both manometers read zero. In 3 subjects, only the abdominal pneumograph was used. Measurements were made on the record from the points where this procedure was followed. The results so obtained were occasionally somewhat erratic probably because other circulatory changes were introduced in the effort to return the diaphragm to a fixed position. Thus, in one subject, expiration during pressure breathing made the feet heavier rather

TABLE 2. *Effect of pressure breathing (PB) on the volumes of the lower leg and the total balance of the body*

SUBJECT	BODY WGT. (a)	VOL. OF FOOT (b)	EFFECT OF PB Δ vol. (c)	EFFECT OF PB Δ torque (d)	PRESSURE OF AIR BREATHED (e)	DISTANCE (f)	CALCULATED TORQUE (g)	DIFF. IN TORQUE (h)	BLOOD TO AB. (i)
	kgm.	*% of wt.*	*cc.*	*kgm. cm.*	*cm.* H_2O	*cm.*	*kgm. cm.*	*kgm. cm.*	*grams*
Fenn	75	3.85	17.0	20.4	30	64	13.1	7.3	292
			15.1	24.8[1]	30	64	11.7	13.1	524
			7.7	11.5[1]	20	64	6.0	5.5	220
			3.8	7.3[1]	10	64	2.9	4.4	176
			15.0	12.1	20	64	11.5	.6	24
Chadwick	72	4.2	6.6	10.6	30	67	5.4	5.2	208
Hegnauer	63	4.0[2]	15.2	24.0	30	68	12.5	11.5	460
Rahn	72	4.2	11.1	31.1	26	77	10.3	20.8	830
Epstein	79	4.2	5.0	11.5	20	65	3.9	7.6	304

Column (b) was determined by filling the plethysmograph with a measured volume of water, with and without the foot. Column (f) is an approximate estimate from the dimensions of the subject and represents the distance from the nipple line to a point on the thigh which is 0.62 the distance from the hip joint to the knee joint. Column (g) is column (c) × 1.05 (density) × 11.5 × column (f). Column (h) is column (d) minus column (g) or the torque not accounted for by blood in the lower half of the body. Column (i) is column (h) divided by 25, and represents the amount of blood which would have to move from the thorax to the abdomen (assumed distance—25 cm.) to explain the remainder of the observed torque, column (h).

[1] Abdomen tightly bandaged—successive measurements in the same experiment.

[2] Estimated.

than lighter. In any event, the method showed us that the results which we have recorded were not due merely to changes in the position of the diaphragm.

The results of these experiments are shown in table 2 for 5 subjects. With pressure breathing, there was in every case an increase in the volume of the foot and an increase in the weight of the foot-end of the board. A typical record is shown in figure 3. In general, the change in torque is greatest in those subjects in whom the changes in foot volume were greatest. Considering only those cases in which a pulmonary pressure of 30 cm. of water was used, it may be said that, on the average, the foot increased 13.5 cc. in volume while the torque in the footward direction increased 19.9 kgm. cm. There was, in other words, a change of torque of 1.5 kgm. cm. for each additional cc. of blood in the foot.

When the balance was disturbed by simply blowing up the cuff without any pressure breathing (data of table 1), the increase in torque was only 0.46 kgm. cm. per cc. of blood in the foot, and this is the change in torque to be expected if, in pressure breathing, the redistribution of blood is confined to a movement of blood from the thorax to the leg below the level of the cuffs. Evidently in pressure breathing some blood also moves into regions of the body between the cuffs and the diaphragm. In order to estimate this quantity of blood, it is assumed that the foot volume indicates the amount of blood per liter of tissue which is displaced into all of the body lying below its center of gravity. According to Cotton (3), the center of gravity in men is located at a point 56.7% of the distance from the sole of the foot to the top of the head. In 4 of our subjects it was found that the volume of the foot in the plethysmograph in cubic centimeters averaged 4.1% of the body weight in grams. Taking 1.06 as the

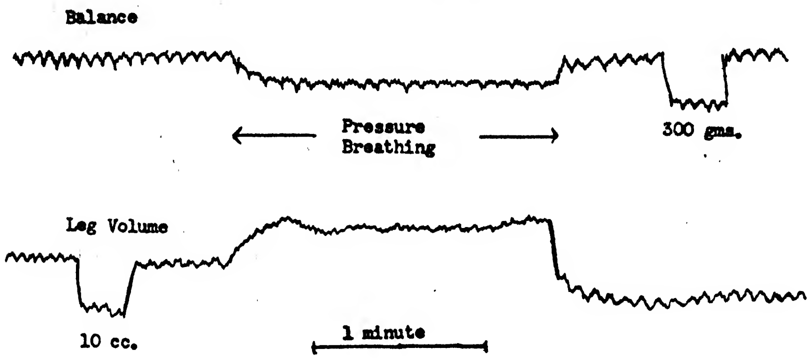

FIG. 3. PRESSURE BREATHING increases the weight of the feet (*fall in upper record*) and increases the volume of the foot in the plethysmograph (*rise in lower record.*). Calibrations are shown on both records.

density of the foot, it may be assumed that the change in blood in the lower 50% of the body is $50/(4.1 \times 1.06) = 11.5$ times as great as that in the foot plethysmograph alone. It may be assumed further that this blood comes from the thorax as it did in the experiments with the cuff alone. To estimate the distance it has moved, it is necessary to determine the location of the center of gravity of the lower half of the body. Using data for the centers of gravity of the limbs and their relative weights given by Fischer (4), we have calculated that, on the average, the center of gravity of the lower half of the body lies at a point which is 0.62 of the distance from the hip to the knee joint. The distance from that point to the mid-thoracic region averages about 69 cm. in our subjects. The change in torque to be expected, therefore, when 13.5 cc. of blood goes into the plethysmograph during pressure breathing is $13.5 \times 1.05 \times 11.5 \times 69$ or 11.2 kgm. cm. The difference $(19.9 - 11.2)$ or 8.7 kgm. cm. must represent blood moving from the thorax to the abdomen.

In order to arrive at an estimate of the volume of blood moving from the thorax

into the abdomen, it is necessary to assume some mean figure for the distance between these two localities. This is difficult to do with any precision, but, for the sake of argument, we have used a figure of 25 cm. The amount of blood will then be 8.7/25 or 348 grams (332 cc.). With these assumptions it may be concluded, therefore, that in pressure breathing with a pressure of 30 cm. of water, 13.5×11.5 or 155 cc. of blood moves from the thorax into the lower half of the body including the legs, buttocks, and lower abdomen while 332 cc. moves into the abdomen.

In addition, some blood presumably goes into the head and arms. That which goes into arms would not change the torque very much because the arms lie at about the same level as the thorax, but blood going to the head would diminish the measured caudad torque. The head represents 7.06% of the body weight, and the foot in the plethysmograph about 4.1×1.06 or 4.35%. If the head received as much extra blood per liter of tissue during pressure breathing as the foot then the change of blood in the head would amount to $\frac{7.06 \times 13.5}{4.35}$ or 22 cc.[2] In order not to change the total torque measured during pressure breathing, it may be assumed that an equal amount of blood moved toward the abdomen an equal distance or, better, that $\frac{1}{3}$ of this volume or 7 cc. moved toward the legs 3 times as far. In addition to this $22 + 7 = 29$ cc., the arms, representing together 13% of the body weight, would receive $\frac{13 \times 13.5}{4.35}$ or 42 cc. Considering the head and arms together with the abdomen and legs, therefore, the total blood displaced from the thorax probably amounted to $155 + 332 + 29 + 42$ or 558 cc. (plus or minus perhaps 200 cc.).

In one individual a series of different pressures was tried (see table 2). The data are insufficient for quantitative purposes, but it seems likely that the amount of blood displaced will be more or less proportional to the pressure. In this same experiment, the abdomen was tightly bandaged so that abdominal breathing was difficult, if not impossible. This was done to avoid artifacts due to variations in the position of the diaphragm. Thoracic breathing by itself has little effect upon the balance of the body, but may tip it headwards slightly during inspiration. The results of this experiment, however, were in no way different from those obtained without such a bandage. In one other individual, in a preliminary experiment not listed in the tables, the abdominal binding was also used with identical results. We feel convinced, therefore, that our results are not due to the displacement of the viscera by pressure breathing. This possibility is also ruled out by the precautions which we took to return the diaphragm to its normal position in taking our readings.

The water plethysmograph. This estimate which we have made of the amount of blood displaced from the lungs suffers somewhat from the assumption that

[2] This is certainly not a strictly valid assumption because the head is largely inextensible, but some blood must go toward the head and accumulate in the face, scalp, neck or shoulders, and this calculation will at least serve to show the order of magnitude of the blood involved in this process.

the foot is representative of the whole lower half of the body. In an attempt to check the validity of this point, we arranged to measure directly the increase in volume of the lower half of the body by immersing the subject in water up to the waist in an oil drum. To increase the sensitivity, a plaster of Paris cover was made which occupied most of the free surface of the water so that the addition of 100 cc. of water raised the total level by about 2 mm. The water level was observed in a glass siphon tube and scale hanging over the edge of the drum. Unfortunately, it was found impossible to include the abdomen below the water level without introducing serious disturbances due to changes in the volume and shape of the abdomen when pressure breathing was initiated. We had to content ourselves, therefore, with immersion of the legs up to the groin.

TABLE 3. *Leg volume in barrel during pressure breathing*

SUBJECT	TEMP. OF WATER	PB	VOL. OF LEGS IMMERSED	Δ WATER LEVEL		Δ VOL. OF BLOOD	Δ BLOOD	
				100 cc.	PB		Barrel	Boot
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
	°C.	*cm. H_2O*	*liters*	*cm.*	*cm.*	*cc.*	*cc/liter*	
Fenn	21	30	22	.27	.21	78	3.5	5.5
Rahn	21	30	18	.18	.03	17	.9	3.7
Rahn	35	30	18	.25	.16	64	3.6	
Chadwick	35.6	30	20.7	.21	.067	32	1.5	
Chadwick	28.5	30	20.7	.26	.103	39.6	1.9	
Chadwick	22	30	20.7	.24	.104	43.4	2.1	2.2

Column (b) is the pressure applied to the lungs to cause the displacement of blood. Column (c) is the amount of water which had to be added to the barrel to bring the level back to the same point after the removal of the subject. Column (d) is the increase in level caused by adding 100 cc. of water when the subject was in position, and column (e) is the corresponding rise caused by the onset of pressure breathing. Column (f) is the ratio of (e) to (d) times 100. Column (g) is the ratio of (f) to (c) or the calculated amount of blood which was displaced into each liter of leg below the water line during pressure breathing. Column (h) is a corresponding figure from the boot plethysmograph experiments for comparison.

The results of these experiments are shown in table 3. The changes in level which resulted from pressure breathing at 30 cm. of water were only 1 or 2 mm., but they were uniformly in the same direction and indicated an increase in volume by 2.3 cc. per liter of leg. The magnitude of this increase varied considerably in different individuals. Subject R was tried on two different days. A very small change was noted on the first day with the water in the barrel at about 21°C., but on the second day, at 35°C., the change was four times as large. On subject C, three different temperatures were tried on the same day, and slightly larger values were found at the lower temperatures. The last column in table 3 shows the volume increase in cc. per liter of tissue at comparable temperatures in the boot plethysmograph. These figures are to be compared with corresponding figures in the preceding column obtained by the barrel method which, on the whole, are somewhat smaller in magnitude. This dif-

ference can probably be attributed to the pressure of the water in the barrel method and the vertical instead of the supine posture. In the standing position, less blood is stored in the lungs and more in the feet. On account of these differences in the conditions, the barrel experiments do not offer much assistance in the quantitative interpretation of our other determinations, but there is certainly no indication that the blood which moves into the upper leg during pressure breathing has been underestimated.

Vasoconstriction due to pressure breathing. In all our subjects, we investigated the effects of pressure breathing while the blood pressure cuffs around both legs were inflated to 60 mm. Hg pressure. The results are interesting because they afford very clear and definite evidence of vasoconstriction in the legs as

TABLE 4. *Pressure breathing with cuff inflated*

SUBJECT	Δ VOL. IN BOOT (a)	Δ TORQUE (b)	PB (c)	Δ VOLUME BELOW CUFF (d)	CALC'D. TORQUE (e)	DIFF. IN TORQUE (f)	Δ VOL. ABOVE CUFF (g)	Δ VOL. FROM THORAX (h)
	cc.	*kgm. cm.*	*cm. H_2O*	*grams*	*kgm. cm.*	*kgm. cm.*	*cc.*	*cc.*
Hegnauer	-7.4	6.7	30	-43	-3.4	10.1	296	253
Chadwick	-3.2	6.6	30	-17	-1.4	8.0	234	217
Fenn	-5.0	11.0	30	-29	-2.1	13.1	384	355
Rahn	-4.7	18.2	20	-23	-2.1	20.3	595	572
Epstein	-14.3	9.1	20	-77	-6.2	15.3	450	373
Average	-6.9	10.3		-38	-3.1	13.4	392	354

Columns (a) and (b) are measured values when P.B. is applied after swelling of foot due to inflation of the cuff to 60 mm. Hg is complete. Column (c) is the pressure of the P.B. Column (d) is the product of (a) and 1.05 and the leg/foot ratio from column (c), table 1. Column (e) is the product of (d) and the measured distance from the mid-thorax to the knee as given in column (b), table 1. Column (f) is (b) minus (e). Column (g) is (f) divided by an assumed distance of 34 cm. representing the distance from the mid-thorax to the center of gravity of that part of the body between the diaphragm and the top of the cuff. Column (h) is the algebraic sum of (d) and (g) and represents the net amount of blood displaced from the thorax in the direction of the feet.

a result of pressure breathing, for, at the onset of P.B. with the cuffs previously inflated, the plethysmograph always showed in all our subjects a decrease in volume which averaged 6.9 cc. or about 2.2 cc. per liter of leg (table 4, col. (a), and fig. 2). When the cuff is inflated, the increase of pressure in the central veins cannot be transmitted beyond the cuff so that there is no passive increase in volume to obscure the decrease which occurs because of the vasoconstriction.[3] The vasoconstriction outlasts the pressure breathing when the pulmonary pressure returns to normal, the lag being about 20 or 30 seconds. This same

[3] This is admittedly an assumption. Our attempts to measure the effect of pressure breathing on the venous pressure below the cuff have not been technically successful. It is not likely, however, that the venous pressure could rise much above the level of the cuff pressure without a considerable dilation and decrease in resistance to flow in the vessels under the cuff.

vasoconstriction reflex apparently occurs also when the cuff is not inflated because the immediate decrease in volume of the leg at the termination of pressure breathing is always greater than the increase in volume at the onset of pressure breathing. The difference between these two volumes is usually about equal to the decrease in volume due to pressure breathing applied while the cuff is inflated. The passive inflation of the leg due to the increased venous pressure terminates almost immediately after the pulmonary pressure falls to normal, and then the vasoconstrictor tone which still persists can cause a further temporary decrease in volume before the vasoconstriction disappears and the leg volume returns to normal. This reflex decrease in volume[4] may possibly represent a constriction of the veins which would serve to raise the venous pressure to compensate for the increased pulmonary pressure. The afferent impulses might originate from the auricle or large veins. A constriction of arterioles, however, cannot be ruled out as an equally possible explanation.

From the change in torque observed during pressure breathing with the cuff inflated (table 4, col. (b)), it is possible to estimate the amount of blood which left the thorax under these conditions. To do this it is necessary to make allowance for the calculated change in torque due to the vasoconstriction in the legs. This has been done in table 4, and the last column shows that, on the average, 354 cc. of blood left the thorax and accumulated in the abdomen and buttocks. This is in fair agreement with the estimate of 332 cc. which was reached from the data of table 2.

In conclusion, it may be said that the experiments with the cuffs inflated are of interest because they demonstrate directly that pressure breathing can still increase the caudad torque due to displacement of blood toward the feet even when movement into the legs is precluded by pneumatic cuffs. This proves directly that there is some movement of blood into the abdomen.

Torque due to pressure breathing with and without venous return occluded. Still another basis for estimating the amount of blood displaced from the lungs is furnished by a comparison of the torques produced by pressure with and without the cuffs inflated, i.e., by a comparison of the torques of table 4 with those of table 2. The pertinent data are included in table 5.

It is evident that in all 5 subjects the change in torque is appreciably greater when the cuff is not blown up (cf. col. (a) and (b)). These changes in torque represent shifts of blood which occur in addition to those shifts due to merely blowing up the cuff. They are not complicated, therefore, by removal of blood from under the cuff itself.[5] On the average, the measured change in torque was 18.4 kgm. cm. without the cuff and only 10.3 kgm. with the cuff. The difference is 8.1 kgm. cm., and this must represent the effect of blood displaced from the

[4] A similar decrease in volume might conceivably be produced by a fall in arterial blood pressure but our measurements (5) have shown that the blood pressure is increased by pressure breathing.

[5] More recent experiments by Dr. Vincent deLalla have shown that a sudden inflation of the cuffs to a pressure above the systolic level causes an increase in the weight of the head-end of the teeter board. This is of the right order of magnitude to be due to cephalad displacement of blood under the cuff itself.

thorax to the legs below the cuffs. If this distance be taken as 76 cm. as an average, then the amount of blood moved is 106 grams or about 6 cc. per liter of leg. This figure is in reasonably good agreement with the values measured in the plethysmograph, i.e., 13.5 cc. in about 3 liters of foot or 4.5 cc. per liter.

Subsequent experiments. After the completion of the original report of this work some further experiments of a similar sort were tried, partly by students in our physiology course and partly by Mr. M. Brontman. These experiments confirmed the original findings qualitatively so far as the plethysmograph was concerned; the teeter-board records were mostly unsatisfactory technically in the few cases in which they were attempted. Mr. Brontman listed 19 records

TABLE 5. *Pressure breathing with and without a cuff on the legs*

SUBJECT	TORQUE DUE TO PB			DISTANCE	Δ BLOOD BELOW CUFF	Δ BLOOD ABOVE CUFF	TOTAL BLOOD FROM THORAX
	With cuff (a)	Without cuff (b)	Difference (c)	(d)	(e)	(f)	(g)
	kgm. cm.	*kgm. cm.*	*kgm. cm.*	*cm.*	*grams*	*grams*	*grams*
Hegnauer	6.7	24.0	17.3	81	214	195	409
Chadwick	6.6	10.6	4.0	84	48	192	240
Fenn	11.0	20.4	9.4	74	127	320	447
Rahn	18.2	25.4	7.2	90	80	530	610
Epstein	9.1	11.5	2.4	81	30	267	297
Average	10.3	18.4	8.1	82	100	301	401

Column (c) is the difference between columns (a) and (b) and represents the Δ torque due to blood moved from the thorax to the leg below the knee. The mean distance this blood presumably moved is shown in column (d) taken from column (f), table 1. Column (e) is column (c) divided by column (d) and measures the weight of blood moved from the thorax to the legs. Column (f) is column (a) divided by an assumed distance of movement of 34 cm. which is a rough estimate of the mean distance from the thorax to the midpoint of the abdomen and buttocks, or that part of the body between the diaphragm and the cuffs on the legs. This represents the weight of blood moved from the thorax to the abdomen and hips, and explains the torque measured during pressure breathing when blood cannot accummulate in the legs because of the inflated cuffs. Column (g) is the sum of columns (e) and (f).

of pressure breathing at 15–20 cm. H_2O on 7 different subjects with a cuff on the leg above the plethysmograph at a pressure of 30–60 mm. Hg. All of these showed vasoconstriction varying from 2 to 50 cc. with an average of 5.5 cc. This agrees well with the figure of 6.9 cc. reported in table 4. In this series, in most cases a vasoconstriction was also obtained without the pneumatic cuff. This would indicate a decrease in volume in spite of the passive dilation expected from the increased venous pressure.

This discrepancy is possibly due to winding the cuff too tightly around the leg so that it protects the leg from passive dilation even before it is inflated. This also may occur when the trousers are rolled up too tightly above the knee. In any event all these experiments confirm the existence of a peripheral vasoconstriction in the foot when pressure breathing begins.

Discussion. These experiments and calculations are only approximations, but we feel that all important errors and artifacts have been eliminated so that the values reported are of the right order of magnitude. Due to errors involved in guessing distances moved, the actual amount of blood displaced may be in error by perhaps 25% or more, but, in round numbers, the measurements show that in the supine position pressure breathing at 30 cm. of water drives 500–600 cc. or 8–10% of the blood volume out of the lungs, about 3% of the blood volume going into the extremities and the remainder into the abdomen.

According to Best and Taylor (6), the lungs normally contain 9% of the blood volume during inspiration and 6% during expiration, but Kuno (7) has shown that they may contain as much as 20% under special conditions. The supine position offers a favorable condition for such an accumulation of blood in the lungs, and Cotton (3) has shown that for 20 minutes after assuming this position the weight of the cephalic end of the body continues to increase as blood returns to the thorax from the legs. The change in torque due to this process which Cotton observed in a supine subject after staircase running was 32.5 kgm. cm. In comparison with this figure, our value of 19.9 kgm. cm. for the change of torque due to pressure breathing seems not excessive. If this estimate is correct, it seems reasonable to suppose that pressure breathing in the supine position at 30 cm. of water could displace half of the blood in the lungs or about 10% of the total blood volume.

The vasoconstriction which we have observed by the boot plethysmograph in a limb protected from passive volume changes by an inflated cuff affords a most objective confirmation of the deduction made from the decreased blood flow in the finger which we have reported (2). Whether this vasoconstriction is a prolonged or merely a transient phenomenon coincident with the onset of pressure breathing is not determined by these experiments. The technique used for this observation is worthy of emphasis because so many vasoconstrictor effects of drugs or other procedures may likewise be obscured by passive venous pressure changes and this offers a ready means of separating these effects.

SUMMARY

Simultaneous measurements were made during pressure breathing of the increase in volume of the lower leg which was enclosed in a plethysmograph and of the increase in weight of the caudal end of the body as indicated by the change in balance of a teeter-board on which the subject lay supine. From these results it may be calculated that an increase of pulmonary pressure of 30 cm. of water displaces 500 cc., or about half of the blood contained in the lungs; this represents about 8 to 10% of the total blood volume. About 3% of the total blood volume goes into the extremities and the remainder into the abdomen. In the standing position, there is less blood in the lungs, and the amount which can be displaced by pressure breathing is correspondingly less.

By the use of a boot plethysmograph it is shown that the onset of pressure breathing causes an increase in foot volume due to passive inflation of the veins. If these passive changes are avoided by the previous inflation of a pneumatic

cuff at 60 mm. Hg placed on the leg above the plethysmograph then the onset of pressure breathing causes a decrease in the volume of the leg due to vasoconstriction.

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EFFECT OF PRESSURE BREATHING ON BLOOD FLOW THROUGH THE FINGER

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Received for publication September 12, 1947

This work to be reported in this paper was done in 1942[1] at a time when positive pressure breathing (PPB) showed promise of military importance as a means of raising the alveolar oxygen tension at high altitudes. Although the results are not so complete as might be desired it seems worthwhile to put them on record because of the novelty of the recording method used and the general physiological implications of the results, which indicate a vasoconstriction during PPB.

METHOD. The finger plethysmograph (fig. 1A) consisted of a light brass sleeve, *b*, terminating at one end in a $\frac{3}{16}$-inch tube and fitting loosely at the other end over the two distal phalanges of the finger. This sleeve was made airtight against the finger by zinc ointment. Volume changes were recorded by a sensitive optical tambour, *c*, and were easily calibrated by a syringe connected into the system by a T-tube. In order to record blood flow by Burton's method (1) a pneumatic cuff, *a*, was adjusted over the base of the finger. This cuff was inflated suddenly by opening the connection to a 2-liter reservoir filled with air at the desired pressure. Since the pressure in the cuff is not necessarily the pressure which is applied to the subcutaneous tissues, the proper pressure was selected by trial as the pressure which caused the maximum rate of filling of the finger. The cuff was made from a brass tube slightly larger in diameter than the finger and about $\frac{5}{8}$ inch wide (fig. 1B). Into a hole through one side of this tube was soldered a short piece of brass tube $\frac{3}{16}$ inch in diameter. A tube of thin rubber about the diameter of the brass ring was passed through the ring and the ends were turned back over the outside of the brass ring on both sides and fastened securely with adhesive tape. The annular space between the rubber tube and the brass ring could then be inflated through the small side tube and so that its pressure was applied to the finger inside.

In using this apparatus we found that inflation of the cuff produced an artifact which appeared as an increase in finger volume even when all blood flow in the arm was stopped by a pneumatic cuff around the upper arm inflated to a pressure above the systolic level. For quantitative purposes therefore it was necessary to evaluate the cuff artifact in this way during complete stasis and subtract this correction from all the observed results. In practice we used an average of 20 such determinations.

[1] This work was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of Rochester. See Report No. 111 under Committee on Aviation Medicine, submitted December 18, 1942.

Measurement of the blood flow was facilitated and made graphic by the device shown in figure 1A. This is essentially a method of obstructing the recording light beam a given interval of time after the pressure is admitted to the cuff so that the height of the excursion in the optical record is a direct measure of the rate of blood flow. When the clamp, *C*, is squeezed this releases the tube, *t*, leading from the pressure reservoir to the finger cuff. At the same time it pulls a pin, *p*, which releases a weight, *w*. The weight sinks slowly in a test tube full of oil or water. After falling a fixed distance it exerts tension on a heart lever, *L*, by means of a thread. On the end of the lever is a flag, *F*, which rises and cuts off the recording beam of light. As soon as the light is obstructed, the operator releases the cuff pressure, opens the plethysmograph to the air, raises the weight and resets the apparatus for another test. These tests can be repeated

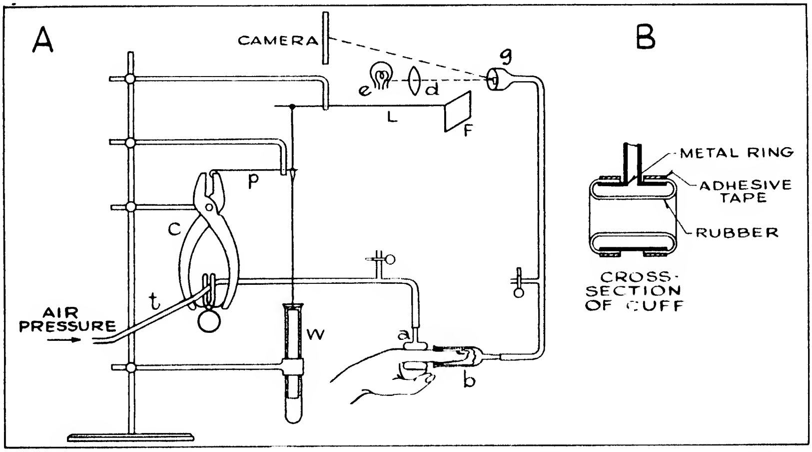

FIG. 1. A. DIAGRAM OF THE APPARATUS used for automatic recording of blood flow through the finger by the plethysmography method. B. CROSS SECTION DIAGRAM of the pneumatic cuff; *a*. See text for further details.

every 15 seconds or oftener if desired. The apparatus was usually so adjusted that the length of the upstrokes measured the amount of filling which occurred during the first few seconds after venous occlusion. The deflections were recorded on a slow drum (0.9 mm. per sec.). The volume flow per second was read from the length of the upstroke (by a calibration table) after deducting the value of the cuff artifact.

During these measurements the subject was seated with the arm resting comfortably on a table at or slightly below heart level. Pressure was applied to the lungs by a pressure helmet closed at the neck by a sponge rubber collar taken from a Drinker respirator. A steady flow of air through the helmet avoided accumulation of CO_2. To increase the pressure the outflow from the helmet was made to pass through a spring-loaded valve set usually for a pressure of 30 cm. H_2O. The pressure helmet was worn throughout the experiment.

Ordinarily 10 or 12 measurements were taken while the subject was breathing under normal pressure, a similar series under positive pulmonary pressure of 30 cm. H_2O, and a final series during recovery at normal pressure. In some experiments, rate of flow was also measured with the subject breathing under normal pressure but wearing an arm cuff inflated to 25 mm. Hg, in order to compare the effects of raised venous pressure *per se* with the effects of positive pressure breathing. In these cases, the measurements with arm cuff inflated followed immediately after the initial period of normal breathing. Each series required about 2 minutes, so that only 6 to 10 minutes were consumed by an entire experiment unless it was desired to test the effects of longer exposures to positive intrapulmonary pressure.

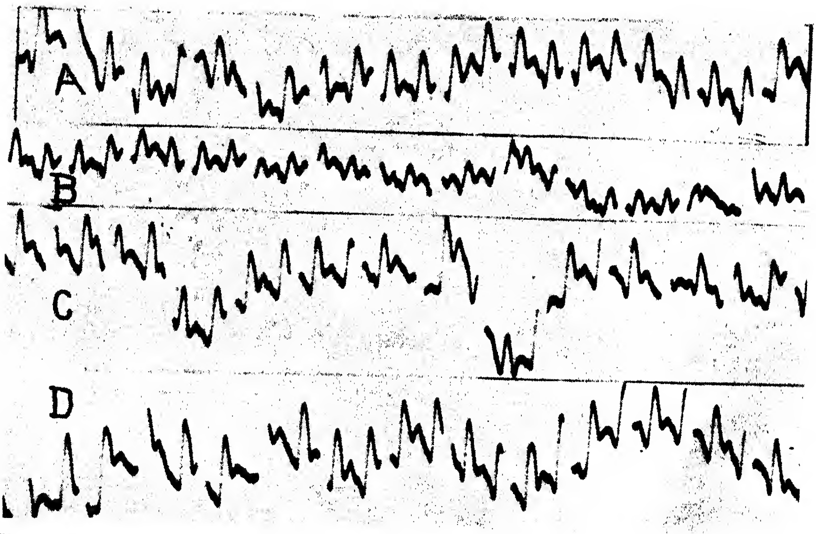

FIG. 2. EFFECT OF PRESSURE BREATHING ON THE FINGER PULSE. *A*. Normal pulmonary pressure. *B*. Beginning of pressure breathing at 30 cm. H_2O. *C*. After 5 minutes of pressure breathing. *D*. Immediately after return to normal pulmonary pressure. Time: gaps in record are at 1-second intervals.

Some of the best results were obtained when the arm was immersed to the elbow in a water bath at 34–35° C. This ensures a considerable and reasonably constant degree of vasodilatation. However, most of our measurements were made at room temperature. Fluctuations in degree of vasodilatation, due to factors other than those which were being studied, probably account for a considerable proportion of the scatter found in our results.

RESULTS. *a*) *Volume pulse*. As may be seen from the typical record presented in figure 2, volume pulse is decreased for a few seconds immediately after positive pressure breathing begins, but usually rises again to a value near the normal level on continued exposure. Upon returning to normal air pressure, the pulse rises above the original normal value for a short time, and then sub-

sides gradually. Individuals vary considerably in the degree of response and in its time relations, and the same individual may give diverse results on different occasions.

b) Rate of blood flow. A typical record obtained with the apparatus shown in figure 1 is shown in figure 3. Each test consists of a short base-line record followed by a sharp upstroke. Between these tests the record is blank while the apparatus was being reset. The exact magnitude of the upstroke varies to some extent according to the particular phase of the cardiac or respiratory cycle. The record shows no essential change when a cuff pressure of 25 mm. Hg was applied to the experimental arm but a sharp and persistent decrease when a pressure of 30 cm. H_2O was applied to the lungs.

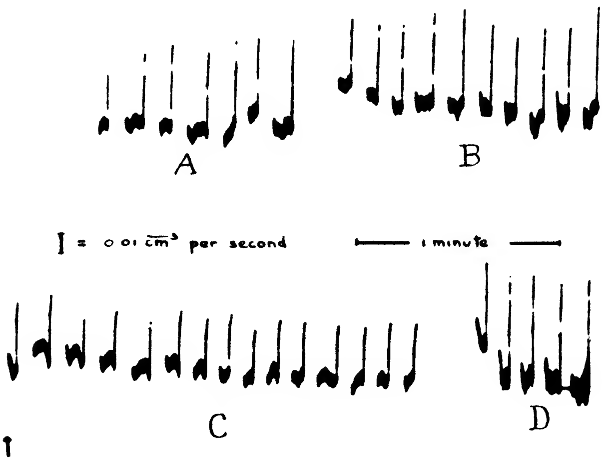

FIG. 3. RATE OF BLOOD FLOW THROUGH FINGER DURING PRESSURE BREATHING. The length of the upstroke represents the rate of flow. *A*. Normal. *B*. Inflation of pneumatic cuff on arm to 25 mm. Hg. *C*. Pressure breathing at 30 cm. H_2O beginning at arrow. *D*. Immediately after return to normal pulmonary pressure.

The results of 18 experiments are shown in table 1. For the sake of simplicity the data are presented in percentages of the normal at the beginning of each experiment. The results can be seen from the averages at the end of the table. Positive pressure breathing decreased the blood flow to 73% of normal with a standard deviation of $\pm 17\%$. Immediately after the return of pressure to normal the blood flow rises to a mean value of 120% of normal with a standard deviation of $\pm 26\%$. The pressure cuff at 25 mm. Hg or 34 cm. H_2O (used in the latter half of the table) decreased the blood flow to only 88% of normal or only half as much as positive pulmonary pressure, in spite of the fact that the cuff increased the venous pressure about twice as much as would be expected from the amount of PPB which was used (i.e. 15 cm. H_2O or about half the pulmonary pressure). It seems evident from this that the decrease in blood flow due to PPB was chiefly due to a vasoconstriction and only to a small extent due to an increased venous pressure.

As indicated in table 1, two experiments were tried in which the PPB was continued for 5 to 7 minutes during which time the blood flow showed no signs of returning to its normal value. With lower pulmonary pressures or in a different individual some recovery might occur as suggested by the recovery of the

TABLE 1. *Comparison of peripheral flow under locally increased venous pressure and during positive pressure breathing (PPB)*

SUBJECT	DATE	PERIPHERAL BLOOD FLOW, IN PER CENT OF NORMAL BEFORE PPB		
		Pulmonary pressure normal with arm cuff 25 mm. Hg	Pulmonary pressure +30 cm. H_2O	Pulmonary pressure normal (recovery)
W. O. F.	11-13-42		82	123
	11-13-42		58	93
	11-13-42		94	125
	11-18-42		51	74
	11-23-42		54	123
W. D.	11-18-42		83	112
L. E. C.	11-17-42		79	114
	11-18-42		96	123
	12- 3-42		86 (74)[1]	106
	12- 3-42		73 (70)[1]	119
W. O. F.	11-22-42	90[2]	71	116
H. R.	11-27-42	64	50	101
L. E. C.	11-25-42	130	64[4]	168
	11-27-42	59	61[4]	129
	11-27-42	87[3]	56[4]	118
	11-30-42	91	57	82
	12- 1-42	81	94	163
	12- 1-42	105	100	170
Mean		88	73	120
Standard deviation		±23	±17	±26
N		8	18	18

[1] Later readings after 5–7 minutes of pressure breathing.
[2] Arm cuff inflated to 20 mm. Hg.
[3] Arm cuff inflated to 30 mm. Hg.
[4] Pulmonary pressure +35 cm. H_2O.

volume pulse shown in figure 2. Occasional records have shown some recovery even during the first two minutes of PPB but on the whole this is not typical of our series.

DISCUSSION. The occurrence of a vasoconstriction in the fingers during pressure breathing is not surprising since a similar reflex is known to result from a single deep inspiration (2, 3). It might be supposed that this results merely

from the expansion of the chest which is common to both PB and inspiration but vasoconstriction has been found to occur in so many respiratory acts (4) that this conclusion is not yet warranted. A diminution in the pulse volume with pressure breathing was observed by Barach *et al.* (5), by palpation, and a transient skin cooling was reported by Bateman and Sheard (6). Further data from our laboratory likewise demonstrate a clear vasoconstriction by a boot plethysmograph method (8) and by skin cooling (to be published). Other related observations are recorded in a recent survey of the subject of pressure breathing (7).

SUMMARY

A method is described by which the rate of blood flow through the finger can be recorded optically every 15 seconds. It is shown that positive pressure breathing results in a decrease in the volume of the finger pulse and a decrease in the rate of blood flow.

We wish to express our indebtedness to our colleagues Drs. Hermann Rahn and Arthur B. Otis who were closely associated with us in this work.

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OXYGEN AND CARBON DIOXIDE TENSIONS OF ALVEOLAR AIR AND ARTERIAL BLOOD IN HEALTHY YOUNG ADULTS AT REST AND AFTER EXERCISE

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Received for publication September 17, 1947

A precise description of the relationship between the tension of oxygen in alveolar air and arterial blood has not been possible because of disagreement concerning methods of collecting alveolar air (1–6) and, until recently, the lack of a suitable technique for direct measurement of arterial blood oxygen tension. It has been demonstrated (7–11) and generally accepted (12, 13) that a state of equilibrium exists between carbon dioxide tension in alveolar air and arterial blood at rest and during exercise. Therefore, when the carbon dioxide tension of simultaneously collected alveolar air and arterial blood samples is the same, the alveolar air sample represents the composition of mean alveolar air. This fact and the recent introduction of methods for direct determination of oxygen (14) and oxygen and carbon dioxide tensions (15) in arterial blood have made it possible to compare the level of oxygen and carbon dioxide in alveolar and arterial blood under different physiological conditions.

The studies herein reported were undertaken late in 1945 on normal individuals to compare with similar observations previously reported in individuals who had been exposed to phosgene (16, 17).

METHODS. *Subjects.* Nine healthy soldiers between the ages of 19 and 25 volunteered for the study.

Experimental conditions. Studies at rest were carried out two hours after breakfast and after a 30-minute rest in bed with one pillow. With nose clip and mouth piece in place, the subject inspired room air. Expired air was collected in a 100-liter spirometer. The rate and volume of respiration were recorded graphically on an ink drum. An alveolar air sample was collected at the end of expiration in an evacuated mercury bottle toward the end of arterial blood withdrawal. The accuracy of the timing of collection of the alveolar air sample was verified by the graphic record of respiration. Arterial blood was drawn anaerobically from the femoral artery under 2% procaine anesthesia into a syringe wet with heparin.

Exercise was performed on a bicycle ergometer and continued for 30 seconds after the subject indicated the onset of definite shortness of breath. The work was at an intensity of from 600 to about 1200 kgm/min. and lasted about 2 to 12 minutes. At the end of work the subject was given the command to dismount the bicycle and place himself in bed with his head resting on one pillow. With-

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drawal of arterial blood was begun promptly from the right femoral artery as during rest and was generally completed within 45 seconds to one minute after the end of exercise. Expired air was collected in a spirometer during the last 30 seconds of exercise and during the period of blood withdrawal. The rate and volume of respiration were recorded graphically during the last 30 seconds of exercise and until the end of blood withdrawal. Alveolar air was collected in an evacuated mercury bottle at the height of inspiration toward the end of blood withdrawal. The accuracy of the timing of collection of the alveolar air sample was verified by the graphic record of respiration.

Analytical techniques. Twenty cc. of blood were drawn through a short beveled 2-inch long, 19-gauge needle into a luer-lok syringe wet with a standard heparin solution (Hynson, Westcott and Dunning) sufficient to eliminate its dead space. This amount of blood permitted duplicate analyses in all instances.

As soon as the blood was withdrawn about 12 cc. were promptly transferred to a tube with a constricted neck, filled with heavy mineral oil and surrounded by ice, for estimation of serum pH and carbon dioxide content. The remaining portion of blood was used for determination of carbon dioxide and oxygen tensions. The pH and the CO_2 content of serum were determined promptly by one individual, and pO_2 (oxygen tension) and pCO_2 (carbon dioxide tension) of arterial blood by another. Gas samples were analyzed later.

The pH determinations were made on serum at room temperature with a glass-sealed electrode (Cambridge Instrument Company). The reproducibility in duplicate analyses is 0.01 pH units. The standardization of the electrode was carried out in two ways. At the start of the study it was done empirically by repeated measurements on plasma from blood equilibrated at 37°C. with gas of known partial pressure of carbon dioxide (18). Later on it was carried out according to the method of Van Slyke *et al.* (19).

The CO_2 content of serum was determined on 1 cc. of serum (20). Calculation of pCO_2 was accomplished on the normogram based on the Henderson-Hasselbach equation from a knowledge of pH and CO_2 content of serum (21). Direct measurements of pO_2 and pCO_2 in blood were made according to the method of Riley (15). Alveolar air and expired air samples were analyzed in duplicate for carbon dioxide and oxygen content in the Henderson-Haldane apparatus.

RESULTS. *pCO_2 in alveolar air and arterial blood.* In 9 observations on 9 subjects at rest the range of alveolar air pCO_2 was 40 to 44 mm. Hg, and of arterial blood 35 to 47 mm. Hg by the direct method and 40 to 46 mm. Hg by the calculated method. The mean alveolar air pCO_2 was 41 mm. Hg, and in arterial blood 42 mm. Hg by the direct method and 44 mm. Hg by the calculated method. After exercise the range of alveolar air pCO_2 in 11 observations in these subjects was 33 to 54 mm. Hg, and in arterial blood 30 to 53 mm. Hg by the direct method and 33 to 52 mm. Hg by the calculated method. The mean alveolar air pCO_2 was 44 mm. Hg, and in arterial blood 41 mm. Hg by the direct method and 41 mm. Hg by the calculated method.

pO_2 in alveolar air and arterial blood. At rest the range of alveolar air pO_2 in 9 observations in 9 subjects was 89 to 103 mm. Hg and of arterial blood 87 to

102 mm. Hg. The mean alveolar air pO_2 was 98 mm. Hg and arterial blood 97 mm. Hg. After exercise the range of alveolar air pO_2 was 98 to 123 mm. Hg and of arterial blood 92 to 118 mm. Hg. The mean level of alveolar air pO_2 was 115 mm. Hg and of arterial blood 110 mm. Hg.

Discussion. Since the direct method for estimating pCO_2 in arterial blood had only recently been introduced and was new in our hands, the results were

TABLE 1. *Summary of Observations*

SUBJECT	STATE	ALVEOLAR AIR pO_2	ARTERIAL BLOOD pO_2	ALVEOLAR AIR pCO_2	ARTERIAL BLOOD pCO_2		SERUM		WORK PER MINUTE	DURATION OF WORK	VOLUME OF AIR BREATHED				O_2 CONSUMPTION	CO_2 OUTPUT
					Direct	Calculated	CO_2 Content	pH			Rest	Work: last 30 sec.	Recovery: first 30 sec.	Recovery: second 30 sec.		
		millimeters of mercury					*vol. %*		*kgm.*	*minutes*	*liters/minute*[1]				*liters/minute*[2]	
1	R	97	94	42	44	43	62.3	7.44								
	E	113	103	43	40	41	49.4	7.34	1040	2.25		41.0	49.6	48.8	1.540	1.750
	E	122	111	42	33	39	45.1	7.32	1245	3.08		52.0	48.4	45.8	1.610	1.880
2	R	98	99	40	40	41	61.0	7.44								
	E	117	112	39	39	37	51.0	7.41	620	12.17		50.4	46.2	43.2	1.580	1.640
3	R	89	87	41	35	43	57.1	7.39								
	R	100	100	42	41	46	58.0	7.36			7.3				0.270	0.240
	E	110	108	48	45	39	48.5	7.35	1245	5.50		48.8	48.6	43.0	1.680	1.835
4	R	103	101	40	41	43	62.4	7.43			12.0				0.425	0.406
	E	119	116	45	41	40	41.3	7.27	1040	5.58		59.2	44.4	46.8	1.810	2.070
5	R	92	95	44	47	46	62.6	7.41			5.4				0.253	0.215
	E	98	92	54	53	52	53.4	7.24	620	6.50		30.2	29.0	26.4	1.340	1.380
6	R	100	101	41	40	46	57.0	7.36			8.0				0.268	0.232
	E	119	118	40	33	36	41.6	7.32	1245	2.50		51.2	51.2	49.4	1.590	1.750
	E	123	117	33	30	33	32.5	7.23	1245	3.58		46.0	43.6	36.2	1.360	1.525
7	R	103	102	40	40	44	56.5	7.38								
	E	122	117	43	40	41	42.1	7.26	1245	2.00		40.4	41.8	40.0	1.510	1.775
8	R	96	94	43	41	40	62.8	7.47			8.0				0.290	0.248
	E	111	104	48	47	45	46.1	7.26	1245	3.58		56.8	59.2	57.0	2.000	2.200
9	R	99	96	44	44	45	62.0	7.42			8.4				0.306	0.278
	E	112	112	47	49	44	45.6	7.27	620	5.75		29.0	26.8	26.0	1.142	1.210

[1] Volumes at 37° C. and prevailing barometric pressure.

[2] Calculated as dry gas at 0° C., 760 mm. Hg, and based on analysis of expired air collected during the last 30 seconds of work and first minute of recovery.

R, resting; E, exercise. All observations were made between 4 Dec. 1945 and 30 Jan. 1946.

checked by the well-established indirect method for calculating arterial blood pCO_2 based on the Henderson-Hasselbach equation (21). At rest the mean alveolar air and arterial blood pCO_2 determined by both the direct and indirect methods were essentially the same (table 1). After exercise the mean alveolar air pCO_2 exceeded that of arterial blood by 3 mm. Hg. This difference may have been due to slight delay in collection of alveolar air samples in some instances.

The close agreement between alveolar air and arterial blood pCO_2 at rest and after exercise is consistent with some previous observations (7–11). It indicates that representative mean alveolar air samples were collected.

The average value of arterial blood pCO_2 reported by Riley *et al.* (23)[2] using the same direct method of analysis is approximately 4 mm. Hg less at rest than observed in this group but is the same during exercise of about the same intensity. In their studies the average carbon dioxide tension of end-inspiratory alveolar air at rest was 4.4 mm. Hg and during exercise 12.7 mm. Hg greater than in arterial blood (22).

As was pointed out previously comparisons of arterial blood and alveolar air pCO_2 were made in the same samples in which oxygen tensions were compared, so as to have a check on whether or not representative samples of mean alveolar air were collected. It will be noted (table 1) that there was consistently close agreement between alveolar air pO_2 and arterial blood pO_2 at rest and to a lesser degree after exercise. The range of pO_2 in alveolar air at rest was 89 to 103 mm. Hg and in arterial blood it was 87 to 102 mm. Hg. The mean value of alveolar air pO_2 was 98 mm. Hg, 1 mm. greater than that of arterial blood. Some previous studies have indicated that alveolar air pO_2 exceeds arterial blood pO_2 by 5 to 25 mm. Hg (24). In a recent study in which an improved method for the direct determination of arterial blood pO_2 was used, the average value of arterial blood and end-expiratory alveolar air pO_2 in young, healthy subjects at rest in a semi-recumbent position was essentially the same (97.1 mm. Hg and 97.4 mm. Hg, respectively) (14). The mean level of 97 mm. Hg in the group reported in this study agrees with the average arterial blood pO_2 of 97 mm. Hg reported recently in healthy subjects (25, 26). These were based on determinations of arterial blood-oxygen saturation.

Riley *et al.* obtained inconsistent values for pO_2 and pCO_2 in end-inspiratory alveolar air samples collected by the Haldane-Priestley method at rest and during exercise (22). They believe that representative samples of alveolar air are not drawn by this method. In an attempt to avoid the short-comings of the Haldane-Priestley method these authors calculate the mean effective alveolar air pO_2 by an indirect method based on the following equation (22):

$$\text{alveolar } pO_2 = \text{tracheal } pO_2 \times \left(\frac{\%\text{ expired } N_2}{\%\text{ inspired } N_2}\right) - \frac{\text{arterial } pCO_2}{\text{expired air RQ}}$$

The concept of mean effective alveolar air gas tension has much to commend it. The equation used involves two assumptions: *a*) mean arterial blood pCO_2 equals mean alveolar air pCO_2 and *b*) respiratory quotient, RQ, based on analysis of expired air equals alveolar air (RQ). The first assumption is supported by previous observations (7–11). There is much dispute about the validity of the

[2] The arterial blood-tension determinations by the direct method involve + 2 mm. Hg CO_2 and − 3 mm. Hg O_2 corrections. These are based on comparisons with tonometer blood-gas equilibration studies. Preliminary comparisons with tonometric standards and the results of determinations by the indirect method for calculating pCO_2 did not indicate to us the need for such corrections.

second assumption at rest, during exercise and deep breathing (1–6, 27–30). The proponents of the opposing view maintain that the expired air RQ is always greater than that of the alveolar air (1, 28, 29, 30).

In comparing alveolar air pO_2 with the mean level of arterial blood pO_2 immediately after exercise or during rest, proper timing of the collection of the samples, consistency of the pattern of respiration and circulatory rate, in addition to the accuracy of the methods of gas and blood analysis, influence the validity of such a comparison. With little training the subjects were able to deliver alveolar air samples as required either at the end of expiration, at rest, or at the end of inspiration, after exercise. The accuracy of timing the collection of the alveolar air sample was checked carefully by the graphic record of respiration in each instance. The respiratory records indicated that the pattern of respiration was quite uniform at rest and during exercise and that after exercise the respiratory minute volume did not fall off significantly during the first and second 30-second periods after exercise while arterial blood was being drawn (table 1). However, the subjects were not in a steady state; O_2 consumption may have been decreasing and CO_2 production by metabolic processes decreasing also. If so, the fact that respiratory volume was maintained would imply either changes in composition of expired air and of alveolar air, of blowing off body reserves of CO_2 or both.

Data on the state of the circulation were not collected during these experiments. However, the close agreement between alveolar air and arterial blood pO_2 and pCO_2 at rest and during the first minute after moderate exercise indicates that the integration of respiration and circulation was good in these healthy subjects.

SUMMARY

At rest oxygen and carbon dioxide tensions of arterial blood determined by the direct method of Riley in 9 healthy subjects were essentially the same as in simultaneously collected alveolar air samples. The mean level of alveolar air oxygen tension was 98 mm. Hg, of arterial blood 97 mm. Hg. The mean level of carbon dioxide tension was 42 mm. Hg in arterial blood and 41 mm. Hg in alveolar air.

After moderate exercise, the mean value of oxygen and carbon dioxide tension in alveolar air was 115 and 44 mm. Hg, respectively, and in arterial blood 110 and 41 mm. Hg.

There was close agreement between arterial blood carbon dioxide tension measured by the direct (Riley) and indirect (based on Henderson-Hasselbach equation) methods at rest and after exercise.

Private Arthur Michalek assisted in carrying out some of the experiments.

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MIXING OF CELLS, PLASMA AND DYE T-1824 IN THE CARDIOVASCULAR SYSTEM OF BARBITALIZED DOGS[1]

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Received for publication August 21, 1947

The time required for injected substances to mix with the entire blood volume must be known if the distribution volumes of these substances are to be interpreted. If material is injected rapidly, it enters the circulation as a short column practically unmixed with blood. Mixing is accomplished largely by the division of this column in vascular circuits with different circulation times and the subsequent combination of the fractions with new volumes of blood at vascular confluences. Large oscillations are observed in the concentration of the injected material in arterial blood until the material is homogeneously distributed in the blood which circulates through the heart, lungs and larger vessels (1, 2). Disappearance of such oscillations does not necessarily mean, however, that the injected substance is now distributed homogeneously through the entire blood volume. As Erlanger has pointed out (3), mixing with the entire volume of blood cannot be complete until the slowest vascular circuit has been traversed at least once. If an appreciable volume of blood is contained in very sluggish channels, material should disappear from the central, rapidly moving circulation into which it is injected, until the slow circuits return as much of the material as they receive. An initial disappearance phase of this sort should be demonstrable in any blood drawn for sampling, since the rapidly moving blood is disproportionately represented in samples drawn from any part of the vascular system.

On the basis of these criteria, the mixing time for injected red cells appears to be of the order of 2 to 4 minutes. After injecting cells tagged with P^{32}, Hahn and Hevesy (4) found that the circulating red cell radioactivity remained constant after $2\frac{1}{2}$ minutes in the rabbit. The maximum time for mixing of cells tagged with radio-iron in the dog appears to be about 4 minutes (5).

In the case of plasma soluble substances, application of these criteria to the study of circulatory mixing is complicated by the fact that such substances do not remain within the blood vascular system. There is thus no achievement of constant plasma concentration to mark the end of the mixing period. Regardless of the nature of the plasma solute which is injected, its concentration in plasma continues to decline, long after the most extravagant allowance for mixing, to complete disappearance. For most of these substances, the rate of disappearance is usually excessive for the first 5 to 25 minutes. The assumption that mixing

[1] Presented in part at the meeting of the American Physiological Society, Chicago, May 18–22, 1947.

with slowly moving peripheral plasma is solely responsible for the excessive early disappearance rate has not been vertified experimentally. Some escape from the blood vascular system during this initial period has been demonstrated for all of the dyes which have been used for measuring plasma volume. The dye T-1824 appears in thoracic duct lymph in detectable concentrations within 15 minutes (6). Smith (7) found the dye brilliant vital red in cervical lymph within 2 to 3 minutes. T-1824 appears quickly in bile (8), and all the dyes are taken up rapidly by the reticulo-endothelial cells (9). Only if these processes remove dye from plasma at a constant rate is it permissible to ascribe the excess rate of disappearance during the initial period to plasma mixing.

In the present studies on circulatory mixing, relatively large volumes were injected, necessitating rather elaborate controls on the effect of the procedures themselves on mixing time. An additional control is afforded by the similarity of the cell-mixing time found with our large volume injections, and the time found by other workers for smaller volumes of tagged cells.

Methods. Dogs, splenectomized soon after the induction of barbital anesthesia, and under anesthesia for at least 3 hours, were used in all the studies.

Cell mixing was studied by injecting cell concentrates in sufficient volume to produce a 15 to 35% increase in the circulating hematocrit and following the change in hematocrit thereafter. The cell concentrates were freshly prepared by centrifuging heparinized dog blood to an hematocrit in the neighborhood of 90%. They were injected in a volume of approximately 10 cc/kgm., as rapidly as possible, through a wide cannula tied in a femoral vein. Usually from 10 to 20 seconds were required for the injection, and time was counted from its completion. For sampling the circulating hematocrit, the brachial artery was cannulated distal to the origin of the profunda brachii. Blood was drawn through needle tubing directly into Wintrobe tubes containing measured amounts of oxalate solution. Before drawing each sample, the system was cleared by bleeding about 0.5 cc., which was approximately double the amount required. The samples were centrifuged for one hour at 2600 r.p.m. in a radius of 20 cm. After deducting the volume of oxalate solution present in each tube, the hematocrits were calculated as 0.92 times the observed reading in order to correct for trapped plasma (10). Hematocrit data were recorded as the ratio cells: total volume in the samples.

The mixing of injected plasma with the circulating cells was studied by following the change in hematocrit with the time after the injection of approximately 10 cc/kgm. of plasma. In order to study the mixing of injected plasma with circulating plasma, one or the other was tagged by the addition to it of the dye T-1824. Samples for plasma dye concentration were drawn from the brachial artery either just before or just after the hematocrit samples. About 2 cc. of blood were drawn for each dye determination into tubes containing dried oxalate. The plasma obtained by centrifuging was diluted 1:11 with a 0.9% NaCl solution, and its optical density in this dilution read against an undyed plasma reference at 620 mμ in a Coleman spectrophotometer. For the present purposes conversion of the optical densities to dye concentrations is not necessary.

Autogenous heparinized plasma was used in all the plasma injections. This was obtained by bleeding the experimental dog soon after the induction of anesthesia and transfusing with blood from a donor animal. *In vitro* mixtures of such plasma with plasma drawn 3 or more hours later, in the presence of dye, gave optical densities which were in good agreement with the expected values. Such agreement was not always obtained when the plasmas of 2 different animals were mixed.

In about half the experiments of each type, expansion of the blood volume by the injection was prevented by withdrawing an equal volume of blood immediately before starting the injection. Blood was drawn for this purpose as rapidly

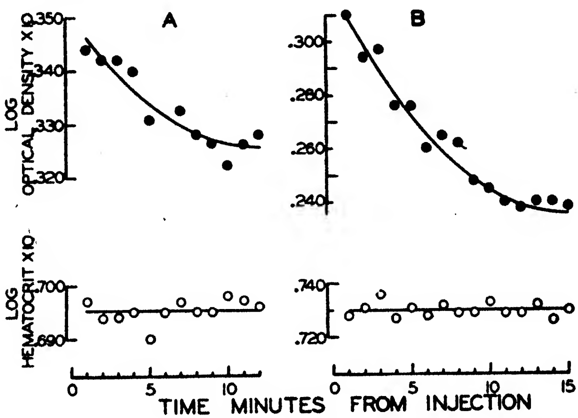

FIG. 1. *A. Dye concentrations and hematocrits following injection of dyed cell suspension.* Intravenous injection at time 0 of 80 cc. cell concentrate containing 10 mgm. T-1824. Control hematocrits average 0.375 (not shown in figure). Line drawn through post-injection hematocrits at average value 0.496 (antilog.). Dye concentration given as optical density against undyed plasma reference. Semilogarithmic plot for both optical density and hematocrit.

B. Consecutive injections of 10 mgm. dye (upper curve) and 100 cc. cell concentrate (lower curve). Construction of figure as in *A*. Average hematocrit before cell concentrate injection equals 0.481, after injection equals 0.537 (antilogs.).

as possible through a large bleeding cannula in the femoral artery. In this modification, the bleeding and subsequent injection occupied less than one minute, and time was counted from the end of the injection.

RESULTS. Representative data on cell mixing are shown in figure 1. In experiment *A* of the figure, dye was added to the cell concentrate before injection in order to obtain simultaneous disappearance curves for dye and cells. It is apparent from the figure that the two curves are totally dissimilar, and that the rapid disappearance of dye from arterial blood is unaccompanied by any similar disappearance of the injected cells. Since the arterial hematocrit was increased about 32% by the injection in this experiment, a progressively declining hematocrit should have been detectable throughout the mixing period

of the injected cells. In experiment *B* the dye and the cells were injected separately, the dye in 10 cc. of solution, in order to make sure that the optical density readings were due to dye alone.

Arterial hematocrits have been taken at one-minute intervals in 11 experiments following injection of cell concentrates. In 6 of these, mixing appears to have been complete within one minute, since the one-minute hematocrit and those immediately following showed no excessive deviation from the post-injection mean, and no progressive change in hematocrit could be detected. In 2 experiments there appear to be cell-mixing periods of 2 and 3 minutes, respectively. In the remaining 3 experiments there was a progressive rise in the hematocrit lasting for 3 minutes. In 10 additional experiments hematocrits have been taken at 3-minute intervals following the injection of cell concentrates, and in none of these is the first value high. The present criteria accordingly show a

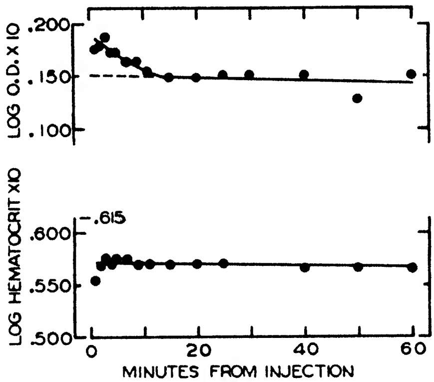

FIG. 2. *Dye concentrations and hematocrits following injection of dyed plasma.* Intravenous injection at time 0 of 100 cc. plasma containing 10 mgm. T-1824. Semilogarithmic plot of subsequent hematocrits (lower curve) and plasma dye concentrations as optical density (upper curve). Pre-injection hematocrits averaged 0.412, post-injection values at 2 to 9 minutes averaged 0.371 (antilogs.).

maximum cell-mixing period of 3 minutes, in a series of 21 experiments. It has not been possible to detect any difference in the experiments in which the injection was preceded by bleeding.

Figure 2 shows the rate of dye disappearance from arterial blood, following the injection of dyed plasma, as compared with the apparent rate of disappearance of the injected plasma. If the excessive disappearance of dye during the first 15 minutes in this experiment is due to loss of the injected dyed plasma to peripheral vascular circuits, a progressive rise in the arterial hematocrit might be expected during this period. No such rise can be seen beyond the second minute. If dyed plasma was lost thereafter, the hematocrit data suggest, accordingly, that the plasma must have carried cells with it in the same ratio of cells:plasma as exist in the central circulation. A total of 14 experiments of this type have been done, half of them with a bleeding preceding the dyed plasma

injection. In addition, hematocrit data have been obtained in 19 experiments after the injection of undyed plasma. In the entire series of 33 plasma injections, the longest progressively rising hematocrit curve lasted for 5 minutes (one ani-

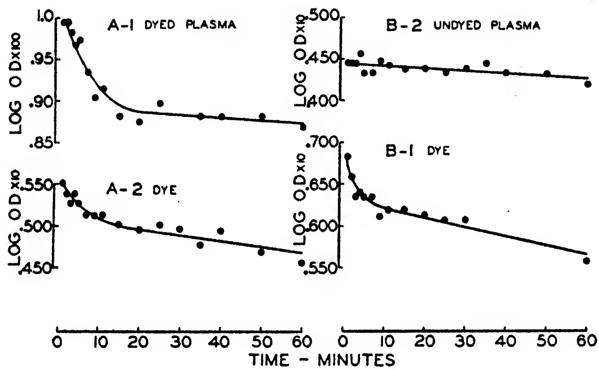

FIG. 3. *A. Dye concentrations following injection of dyed plasma (upper curve, A-1) and aqueous dye solution (lower curve, A-2) in same animal.* Dyed plasma injected in volume of 100 cc., containing 3.5 mgm. T-1824, immediately preceded by 100 cc. bleeding. Aqueous dye solution given one hour later, volume 10 cc., containing 10 mgm. dye. Semilogarithmic plot both curves. Dye concentrations as optical density.

B. Dye concentrations following injection of undyed plasma (upper curve, B-2), in dyed animal. Lower curve, B-1, shows injection of 20 mgm. dye in 20 cc. aqueous solution, 1½ hours earlier (last 30 minutes of curve not plotted). Plasma injected in volume of 90 cc., preceded by bleeding of equal volume. Construction of figure as in *A*.

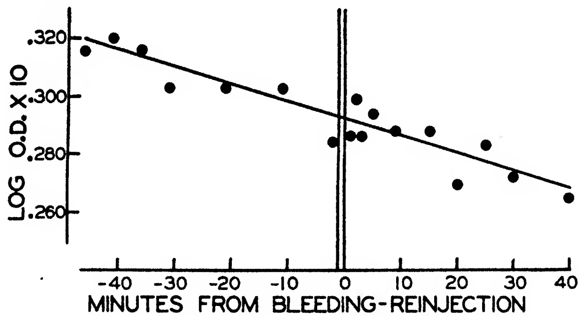

FIG. 4. *The effect of bleeding and reinjection on the rate of dye disappearance from arterial plasma.* Twenty mgm. of dye were injected at minus sixty minutes. At minus one minute approximately 10 cc./kgm. of blood were withdrawn from an artery into an heparinized syringe, and at time 0 this blood was reinjected into a vein. Dye concentrations are plotted semilogarithmically as optical densities for 40 minutes before and after the bleeding and reinjection.

mal). In over half the experiments, such a curve was either absent from the data or terminated within 2 minutes. The average duration of the curve was somewhat longer, in this series, for the injections not preceded by bleeding.

Figure 3 shows the similarity of the disappearance curves for dye when injected as a large volume of dyed plasma and as a small volume of saline solution and the total dissimilarity of the curve obtained when undyed plasma is injected into a dyed animal. If peripheral plasma is dyed by injecting dye into an animal and allowing an hour or more to pass for thorough circulatory mixing, a subsequent injection of undyed plasma should produce a typical mixing curve recognizable as a progressive increase in arterial dye concentration. If the excessive fall in arterial dye concentration following dye injection is due to loss of dyed plasma in the sluggish peripheral circuits, there should be an equivalent period of rising dye concentration following undyed plasma injection, due to dye gain from these same circuits. Curve B-2 in figure 3 offers no suggestion of such reversed mixing. Since curve A-1 for dyed plasma and curve B-2 for undyed plasma were obtained under identical experimental conditions, they should present similar features, in opposite sign, if plasma mixing is a limiting factor. Undyed plasma has been injected into dyed animals in 19 experiments of this sort. In 12 of these, the injection was preceded by bleeding. In most of the latter, the data resemble curve B-2 of the figure, i.e., no curve of rising optical density can be made out if the first sample is drawn at one minute. Such curves could usually be recognized if the bleeding was omitted. The longest duration observed in either type of experiment was 5 minutes.

Discussion. We are unable to reconcile these data with the belief that the excessive disappearance of injected dye from arterial blood during the first 5 to 20 minutes represents movement of dyed plasma from the central to the peripheral circuits. No such movement of undyed plasma can be demonstrated. Undyed plasma, using the same criteria as are applied to dyed plasma, appears to mix completely within a maximum of 5 minutes, usually much less. The mixing time for injected plasma thus appears to be not much, if any, longer than the mixing time for injected cells. The mixing time for injected plasma as obtained from the rising arterial hematocrits, and from the rising plasma densities in the undyed plasma injections, appears to be the same. The average plasma mixing time is about 2 minutes, with a maximum of 5 minutes, in both types of experiment. The hematocrit data in the plasma injection experiments show differential plasma loss from the central circulation. Since they are in good agreement with the optical density data, it seems likely that they show total plasma loss as well.

Since the source of errors in the hematocrit and the optical density data are not the same, agreement between the two data is evidence of the reliability of both. The possibility that our procedures introduce systematic masking errors in the form of peripheral trapping, or a change in the rate of dye disappearance, has been further examined in blank control experiments. In some of these, undyed animals were subjected to bleeding and injection of undyed plasma. Plasma optical densities, read at one-minute intervals thereafter, showed no deviation from the pre-injection values. In other control experiments, animals were injected with dye and allowed to remain undisturbed for periods of $1\frac{1}{2}$ to 2 hours in order to achieve a more-or-less constant rate of dye disappearance (11).

A blank bleeding and injection was then done by withdrawing approximately 10 cc/kgm. of blood from an artery into heparinized syringes and reinjecting at the end of one minute into a vein. Figure 4 shows the type of data obtained in such control experiments. The post-injection dye concentrations show neither an excessive deviation nor a change in the rate of dye disappearance.

The present data suggest not only that the excessive early disappearance of dye is the result of dye-loss from the circulation; the absence of dye-return following undyed plasma injection must mean either that the loss is irreversible or very slowly reversed. Comparison of the rate of dye disappearance before and after undyed plasma injection in figure 3B shows a reduction in the rate after the plasma injection. This phenomenon, which could mean slow return of dye to the central circulation, has another explanation which is given elsewhere (11).

Since circulatory mixing of injected plasma solute appears to require up to 5 minutes in exceptional cases with our large volume injections, it would be important to know the maximum mixing time for the usual small volume of injected dye. Withdrawal of a fairly large volume of blood, and replacement with an equally large volume of dyed plasma, might be expected to shorten the mixing time to a minimum. The period of initial rapid dye disappearance, however, does not appear to be consistently different under these conditions and under the conditions of the usual dye injection (see fig. 3A). The magnitude of dye loss during this period, measured as the difference between the first observed concentration and the exponential extrapolation at the same time, seems to be quite variable, in contrast with the duration of the phase of early disappearance, which varies from animal to animal, but is influenced relatively little by a change in conditions. In some animals, the magnitude of the early loss appears in our experiments to be greater with conventional dye injections, in others with dyed plasma injections. Nor has it been possible to establish a consistent difference in the magnitude of the loss on first and subsequent dye injections.

SUMMARY

The circulatory mixing of cells, plasma and solutions of the dye T-1824 was studied in barbitalized dogs following rapid intravenous injection of cell concentrates, dyed and undyed plasma and aqueous dye solutions. As shown by progressive changes in the arterial hematocrit following the injection, the mixing time for both injected cells and injected plasma was usually less than 3 minutes and never longer than 5 minutes.

More crucial data on the mixing of injected plasma within the entire plasma compartment were obtained by injecting undyed plasma into dogs which had been previously injected with dye. The duration of the mixing period, as shown by a rising arterial dye concentration after the injection, was similar to that shown by the hematocrit studies. Following this brief period, arterial dye concentration continued to fall at an approximately exponential rate as before the undyed plasma injection. When dyed plasma was given in similar volumes and under identical conditions, so as to increase the circulating dye concen-

tration, the concentration of dye in arterial plasma fell rapidly for as long as 20 minutes before achieving an approximately exponential rate of disappearance.

It is concluded on the basis of these data that the time required for circulatory mixing of both cells and plasma is less than 5 minutes under the experimental conditions described. The time for mixing of plasma solute within the vascular system must be the same as that for plasma. The continued rapid disappearance of injected dye beyond the first 5 minutes is, therefore, ascribed to passage of dye outside the plasma compartment.

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RATE OF DISAPPEARANCE OF DYE T-1824 FROM ARTERIAL BLOOD

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Received for publication August 21, 1947

The actual rate at which injected dye leaves the blood vascular system is of no concern for the measurement of plasma volume if the rate is constant. Erlanger (1) suggested that under these circumstances it should be possible to extrapolate from any convenient portion of the curve to the concentration at the instant of injection. In the method developed by Gibson and Evans (2), and currently followed with more or less minor modifications, the exponential, or nearly exponential, rate of decline in plasma dye concentration, which is achieved within 5 to 20 minutes of the injection, is extrapolated for this purpose. Relatively little attention has been given to the faster rate at which dye leaves the central circulation during the first few minutes, since it has been generally assumed that the excessive early rate of disappearance represents circulatory mixing. The present study was prompted by data reported elsewhere which show that the time required for mixing of plasma solute is far shorter than the so-called 'mixing period' for injected dye (3). Since a considerable portion of the dye which disappears from arterial plasma during this early period must accordingly be lost from the circulation, a study of the early rate of dye disappearance is obviously indicated.

Methods and Results. All determinations were made on arterial samples drawn from splenectomized dogs in a steady state of barbital anesthesia. The procedures have been described in full elsewhere (3). Spectrophotometrically stable solutions of the dye T-1824 in a concentration of approximately 1 mgm/cc. in 0.9% NaCl were given by quick intravenous injection. Doses were usually of the order 10 to 20 mgm. and produced plasma dye concentrations between 0.01 and 0.03 mgm/cc.

Figure 1 shows the data from a typical dye injection. The upper curve is a linear plot of the data which can be fitted to a straight line after the first 20 minutes. The middle curve is a semilogarithmic plot of the same data. It lengthens the linear segment to include all the values after the first 12 minutes. The lower curve is a full logarithmic plot which permits all the data for the first hour to be fitted to a straight line. The deviation of the data from this line after the first hour, which is shown in the figure, is typical of all our experiments. The break is always downward, as shown, and comes between 45 and 75 minutes. If the data are plotted either linearly or semilogarithmically for 3 or 4 hours after the injection, it is obvious that under our experimental conditions both types of plot are in reality long curves rather than straight lines. Deviation

from rectilinearity is not, therefore, peculiar to the full logarithmic plot; it is simply more abrupt, more marked and opposite in direction.

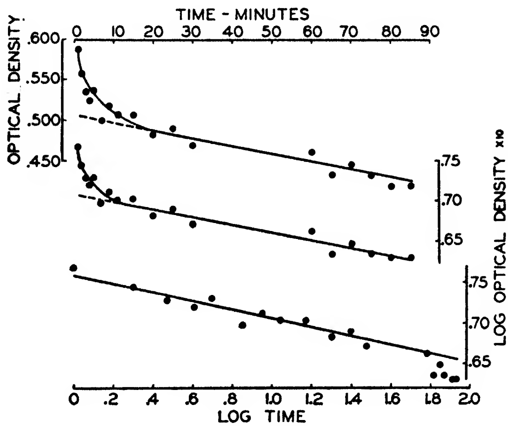

FIG. 1. The uppermost curve is a linear plot of dye concentration, as optical density, following injection of 20 mgm. of the dye T-1824. It is read against the linear scales at the left and above. The middle curve is a semilogarithmic plot of the same data, read as the log. of optical density on the scale at the right against the linear time scale above. The lowermost curve shows the data plotted logarithmically against the logarithmic scales on the right and below.

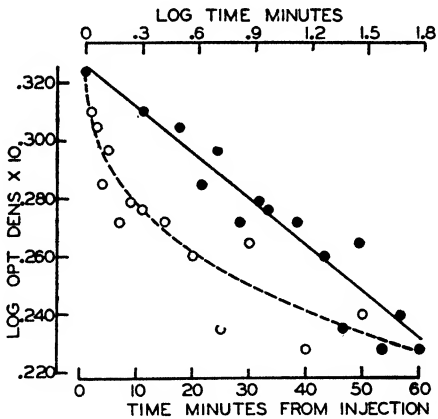

FIG. 2. The filled circles and the solid line are a full logarithmic plot of optical densities following injection of 100 cc. of plasma containing 10 mgm. of dye; to be read against the log. time scale above. The injection was preceded by withdrawal of an equal volume of blood. The open circles and the dotted line are a semilogarithmic plot of the same data, to be read against the linear time scale below.

Straight-line logarithmic plots for the greater part of the first hour have been obtained with all dye injections in our experiments, whether the dye is given as a

small volume of dye solution or as a large volume of dyed plasma. Figure 2 shows an injection of dyed plasma plotted semilogarithmically and logarithmically. In both types of plot, the time-concentration curves appear to be similar to those obtained for the usual dye solutions.

Figure 3A shows a fourth-hour disappearance curve as a straight line on a semilogarithmic plot. Injection of undyed plasma at this time caused a sudden decrease in dye concentration but no detectable change in the disappearance rate as a function of the existing concentration. Results of this sort were obtained when undyed plasma was given several hours after the injection of dye, there being either no change or a very small reduction in the slope of the time-concentration curve.

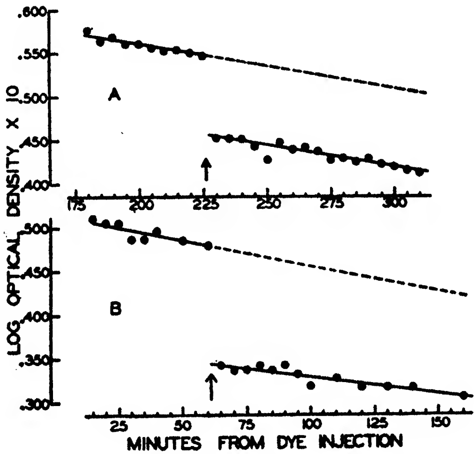

FIG. 3. *A. The effect of undyed plasma injection on the rate of dye disappearance during the fourth hour.* The arrow marks injection of 100 cc. undyed plasma 3¾ hours after the injection of dye. The slopes of optical density have been determined by the law of least squares. The rate of dye disappearance before plasma injection is 6.90% per hour; after injection, 6.89% per hour.

B. The effect of undyed plasma injection on the rate of dye disappearance during the second hour. Experiment and construction of figure as in *A*, except for timing. Rate of dye disappearance before plasma injection is 7.28% per hour; rate after plasma injection is 5.25% per hour.

Figure 3B shows the contrasting results obtained when undyed plasma was injected shortly after the dye. In the experiment illustrated, when the dye concentration was suddenly reduced by an early plasma injection, one hour after the dye had been given, there was an abrupt lessening of the rate of dye disappearance. These observations are in conformity with data obtained by following dye concentrations for periods of 3 to 4 hours in undisturbed animals. During the first 1 to 3 hours following dye injection, the rate of dye disappearance decreases with decreasing concentration. The appearance of rectilinearity on semilogarithmic plots for this period is dispelled by plotting at short

intervals for longer periods, or by suddenly decreasing the concentration with plasma injection.

Figure 4 affords a quantitative comparison of the early dye disappearance rates following a first and a second dye injection. The two 'total' curves in the figure are logarithmic plots of the total dye concentration. The slope of the second injection, when plotted in this fashion, is obviously less than the first. Two hours were permitted to elapse between the 2 dye injections. During the latter half of the second hour, the dye first injected was found to be disappearing at an approximately exponential rate which was plotted out and extrapolated

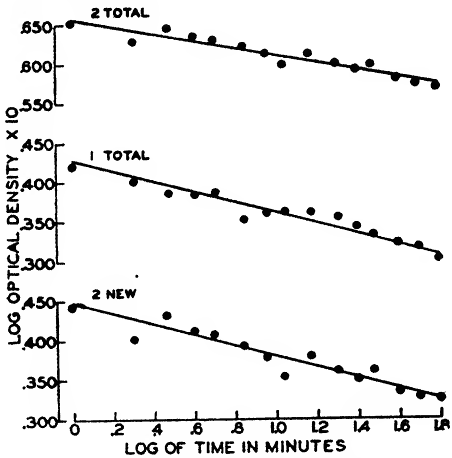

FIG. 4. *Dye disappearance following a first and a second dye injection.* Full logarithmic plots. The middle curve, marked '1 Total.' shows the optical densities following a first injection of 10 mgm. dye. The upper curve, '2 Total,' shows the total optical density following a second 10 mgm. dye injection approximately 3 hours later. In curve 1 Total, dye disappearance is 24.49% in the first hour; in curve 2 Total, the disappearance is only 17.24%. The lowermost curve, '2 New,' is a derived plot for the optical density of the newly injected dye alone. It was constructed by plotting optical density at 5-minute intervals for one hour preceding the second injection, fitting the data to an exponential slope and extrapolating this slope through the period of the '2 Total' curve. This plot and its extrapolation are not shown in the figure. The derived curve is the difference between the '2 Total' curve and this extrapolation. The disappearance rate for the newly injected dye, when distinguished in this manner from the 'old dye,' is 24.25% in the first hour. All lines, including the subtracted extrapolation for old dye, drawn by the law of least squares.

through the third hour. The lowermost curve in the figure was constructed by substracting this extrapolation from the '2 total' curve. This subtraction assumes that the dye first injected, now in its third hour, will continue to disappear exponentially during the period when the newly injected dye is disappearing as a full logarithmic function. When the new and the old dye are distinguished in this manner, their early disappearance rates, as shown in the figure, are practically identical.

Discussion. The relationship shown between dye concentration and time in our full logarithmic plots is:

$$C_t = \frac{C_1}{T^p}$$

where C_t is the dye concentration at time t, C_1 is the concentration at one minute, and T^p is a power, less than one, of time. All time values in this formulation are given in minutes from the injection. The average value found for p in a series of 20 conventional dye injections was 0.0553, with the relatively large standard deviation of $\pm$ 0.0117. It is apparent that this relationship can exist for only a limited period of time. As T approaches 0, the value of C_t approaches infinity. Since the highest possible dye concentration is that of the injected solution, i.e., about 1 mgm/cc., it is obvious that the full logarithmic plot cannot represent the data immediately following the injection. Extrapolation to the moment of injection would require the insertion of arbitrary constants in the formula, a device for which there is no justification in the present data. An attempt has been made in 2 experiments to determine the shape of the dye disappearance curve during the first minute by sampling at intervals of 10 seconds after the injection. Large variations were observed which were interpreted as recirculations of the incompletely mixed column of dye. Most of the values fell above the line representing the later data in terms of the formula above. Further study of the very early disappearance rates is needed.

For the present purposes it is of minor concern that the logarithmic plot also demonstrably fails to represent the data beyond the first hour. In the square root of time formula of King, Cole, and Oppenheimer (4) an empirical time constant is employed to represent the deviation of the later data from rectilinearity. Their formula does not, however, represent the data for the first few minutes, which are assumed to be distorted by a long mixing period. Gellhorn, Merrell, and Rankin (5) obtained a double exponential equation which represented the change in concentration of injected Na^{24} and which could be adapted to the present data. The constants employed in their equation, however, were empirically determined from rates of change observed several minutes after the injection. When the equation was solved for concentration at time 0, calculation of plasma volume yielded values about twice those obtained by dye methods, suggesting that their equation did not describe the reaction during the period immediately following the injection. If, as our data show, the rate of dye disappearance changes with time, extrapolation to time 0 is likely to be extremely unreliable regardless of the method employed. The rates of change in dye concentration which are observed after the first 2 to 5 minutes may be taken to represent dye escape from the vascular system after circulatory mixing is complete. Earlier rates of disappearance from the central circulation must be influenced by mixing, and may be either greater or less for this reason.

Although it does not seem profitable to attempt a formulation of the entire dye disappearance curve, the data of the present report permit certain qualitative statements. (*a*) Any new injection of dye must pass through an initial

phase of rapid disappearance during which the overall rate of disappearance from the central circulation is represented by a straight line on a full logarithmic plot. If the rate at which previously injected dye is disappearing at this time is subtracted (fig. 4), no difference can be detected between a first and a second dye injection. (*b*) This phase is followed by a period lasting 1 to 3 hours during which the rate of disappearance gradually declines with the decrease in concentration. If undyed plasma is injected during this period to produce a decrease in dye concentration, the rate of dye disappearance is decreased (fig. 3B). (*c*) There is a subsequent period during which the rate of disappearance seems to be a fixed percentage of the existing dye. This exponential rate of disappearance is not changed by suddenly decreasing the dye concentration (fig. 3A). We have not studied still later disappearance rates. The identity of behavior of a

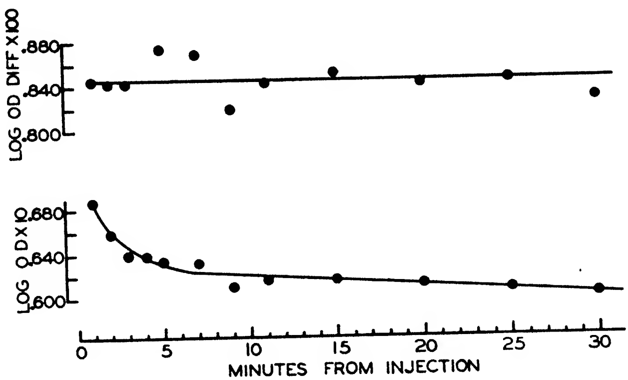

FIG. 5. *The rate of disappearance of injected undyed plasma from the arterial circulation.* The lower curve is a semi-log. plot of optical density following injection of 20 mgm. of dye. The upper curve is a semi-log.plot of the decrease in optical density obtained 2 hours later by injecting undyed plasma. The decreases plotted in this curve were obtained by subtracting from the observed density, on each time ordinate, the density read by extrapolation of the pre-injection values. Approximately a 25% reduction in optical density was produced by the plasma injection.

first and a second dye injection, when allowance is made for the changing rate of disappearance with time, is at variance with the report of Cruickshank and Whitfield (6).

The fact that each new dye injection must pass through a phase of rapid disappearance is, of course, what would be expected if circulatory mixing is responsible for the initial fast phase. But such an initial phase would also be expected if dye is lost from the plasma compartment into a space which reaches a state of equilibrium with plasma fairly soon. If, as the present data show, injecting undyed plasma in the third phase of dye disappearance does not change the rate of dye disappearance, it should be possible to plot the disappearance of the undyed plasma from the central circulation as a curve of dye decrement. Such a curve is shown in figure 5. No loss of the undyed plasma from the arterial

circulation can be detected after the first minute, the deviations from the horizontal being random in direction (exaggerated by the large scale plotting). This is an extension of data given in full elsewhere as evidence that the initial rapid dye-disappearance phase is not due to circulatory mixing (3).

SUMMARY

When concentrated solutions of the dye T-1824 are injected intravenously in barbitalized dogs, arterial dye concentrations from one minute to approximately 60 minutes after the injection decrease in accordance with the relationship $C_t = \frac{C_1}{T_p}$, where C_t is the concentration at any minute following the injection, C_1 is the concentration at one minute, T is time in minutes from the injection and p is a fractional power. The average value found for p in 20 dye injections was 0.0553, standard deviation $\pm$ 0.0117. The straight line obtained for the data when plotted as log. concentration against log. time is useful for smoothing the early data during the phase of rapid disappearance and for quantitative study of the early disappearance phase.

A truly exponential rate of disappearance is not achieved, as a rule, earlier than 2 to 4 hours after dye injection. Whereas undyed plasma injection does not change the percentage disappearance rate if given at these longer intervals, earlier plasma injections reduce the percentage rate of disappearance. Three phases of dye disappearance are thus recognized: *a*) An initial rapid disappearance phase, lasting approximately one hour, in which the log. of dye concentration is a linear function of the log. of time; *b*) a transition phase, lasting 1 to 3 hours; and *c*) an exponential phase, in which the log. of dye concentration is a linear function of time. Each new injection of dye appears to pass through all three phases. The total rate of disappearance for a second dye injection appears to represent simply the sum of the rates for the old and the new dye. When the disappearance rate for the previously injected dye is subtracted from the total, no difference can be detected between a first and a second injection.

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MEASUREMENT OF PLASMA VOLUME AS THE DISTRIBUTION VOLUME OF INJECTED AUTOGENOUS PLASMA

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Received for publication August 21, 1947

If the initial excessive rate of disappearance of injected dye does not represent circulatory mixing, but a time-limited loss from the vascular system (1), the volume obtained by the method of Gibson and Evans (2) is obviously the volume of plasma plus a volume related to the space occupied by the escaped dye. That the method of Gibson and Evans overestimates the plasma volume is suggested by a variety of observations (3, 4, 5). In none of these studies, however, is a more reliable, independent estimate of plasma volume offered for comparison.

If the current dye injection methods are valid for estimating plasma volume, similar values should be obtained from the injection of undyed plasma into dyed animals and noting the decrease in dye concentration. It is shown elsewhere that such plasma injections do not change the rate of dye disappearance, if given at a sufficiently long interval following the dye injection (6). Dye which has been lost from the plasma compartment does not, accordingly, appear to return at an appreciable rate when undyed plasma is given. If the dye injection method is in error due to dye escape, there should therefore be no comparable error due to dye return when the volume is measured by injecting undyed plasma. In the latter procedure, no assumptions have to be made regarding the actual amount of circulating dye, except that it is not changed by the plasma injection.

The dilution of normal plasma constituents by injection of large volumes of crystalloidal or foreign colloidal solutions has been employed before for measuring plasma volume(7, 8). In addition to the large technical errors inherent in the methods, the injected solutions could not be relied upon to remain at the injected volume within the circulation. In the present study, percentage technical errors were reduced by injecting enough dye to give a high optical density to the circulating plasma and by using undyed autogenous plasma for the subsequent dye dilution, in sufficient volume to give a 15 to 30 % decrease in circulating dye concentration. The autogenous plasma used for the dilution should undergo minimum changes in volume within the circulation.

Method. Barbitalized, splenectomized dogs were used. Details of all the experimental procedures have been given elsewhere (1). Autogenous heparinized plasma was obtained by bleeding and transfusing with donor blood, the animal being allowed to rest for about 3 hours after the transfusion before starting the experiment. In dying the c rculating plasma, the dose of T-1824 injected was usually 20 mgm., and the resulting dye concentrations were of the order 0.025 to 0.035 mgm/cc. The undyed plasma was injected from 1 to 4 hours later in a volume of approximately 10 cc/kgm.

The calculation of plasma volume is based on the assumption that the optical density of the mixture of dyed and undyed plasma in the circulation after injection of the latter is simply the mean of the two densities as measured before the injection. Under these conditions

$$PV = \frac{V_r \times (E_m - E_r)}{E_p - E_m} + P_e$$

where PV is the volume of circulating plasma at the instant the injection is started, V_r is the volume of injected plasma, E_p is the optical density of the circulating plasma before the injection, E_m is the density of the mixture after the injection and E_r the density of the injected plasma. If all the optical densities are read against an undyed plasma reference, and autogenous plasma is used, E_r drops out of the equation. If the plasma injection is preceded by

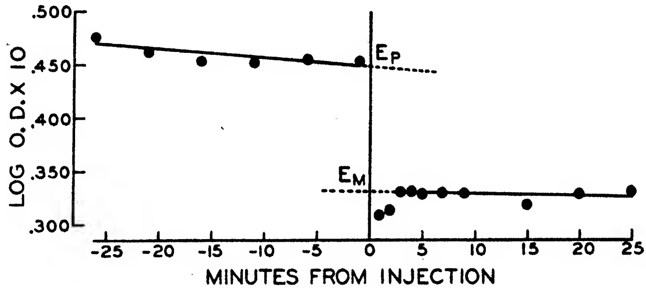

FIG. 1. *Measurement of plasma volume by dye decrement.* At time 0, 100 cc. undyed plasma were injected following withdrawal of 100 cc. blood. The extrapolations yielded E_p = 0.2806 and E_m = 0.2143 (anti-logs.). Since the optical densities were read against undyed plasma as a reference, $E_r = 0$. Plasma volume (see text) is thus: $PV = \frac{100 \times 0.2143}{0.2806 - 0.2143}$ = 323 cc. In this experiment there appears to be a 3-minute mixing period for the injected plasma.

bleeding, in order to prevent expansion of the blood volume by the injection, P_e is required in the equation to show the volume of plasma withdrawn in the bleeding. Figure 1 shows the type of data obtained. E_p and E_m are read at the completion of the plasma injection by extrapolation of the two optical density curves. When the plasma injection was not preceded by bleeding, the post-injection densities often appeared to fit a complex function, making extrapolation to the instant of injection difficult. In most of the experiments of the present report we have accordingly withdrawn an equal volume of blood just before the injection of plasma.

Results. The essential data are given in table 1, which shows comparative values for plasma volume obtained as follows: (*a*) By the dye injection method of Gibson and Evans, as $PV = \frac{D}{c'}$, where D is the amount of dye injected and c′ is the concentration at time 0 obtained by extrapolating the approximately

exponential segment of the time-concentration curve plotted after the first 15 minutes. (*b*) By the same formula, substituting for Gibson and Evans' c' the dye concentration read at one minute on the log.-log. smoothed plot previously described (6). This value will be referred to as the one-minute dye volume. (*c*) By the subsequent injection of undyed plasma as described in the foregoing. This value will be called the dye-decrement volume.

The table shows that the apparent distribution volume of the dye, at one minute following its injection, is usually very similar to the permanent distribution volume of injected undyed plasma. Both of these volumes are consistently smaller than the plasma volume as obtained by the method of Gibson and Evans. Where there is any appreciable difference between the one-minute dye volume and the dye-decrement volume, the one-minute volume is always the larger. This would be expected if some dye has escaped from the plasma compartment within the first minute.

TABLE 1. *Comparative values for plasma volume*

The values given under the heading *G & E* were obtained with the dye method of Gibson and Evans. The column headed *1-min. dye* gives values obtained by substituting the concentration of dye observed at one minute in the Gibson and Evans' calculation. The values listed under *Dye decrement* were obtained with undyed plasma injection as described in the text. The last 3 columns give the ratios of these comparative values. Dye decrement values for *dogs 11* and *12* were obtained without bleeding before the injection, all others with bleeding (see text). All volumes are given in cubic centimeters.

DOG NO.	G & E	1-MIN. DYE	DYE DECREMENT	1-MIN/G & E	DYE DEC/G & E	1-MIN/DYE DEC.
1	414	370	363	0.894	0.878	1.019
2	500	454	411	0.907	0.823	1.102
3	470	395	361	0.840	0.768	1.094
4	650	567	570	0.872	0.876	0.994
5	552	491	490	0.890	0.888	1.001
6	370	341	345	0.921	0.933	0.989
7	346	308	296	0.890	0.856	1.039
8	576	498	466	0.866	0.808	1.069
9	380	338	335	0.890	0.882	1.008
10	489	410	385	0.840	0.788	1.064
11	620	534	532	0.861	0.858	1.004
12	545	446	454	0.818	0.833	0.984
Average				0.874	0.849	1.031

Figure 2 shows the agreement between the one-minute dye volume and the dye-decrement volume in another form. If Gibson and Evans' interpretation of the time concentration curves for dye is correct, the distribution volume of dye within the vascular system is expanding throughout the initial phase of fast disappearance. In the construction of figure 2 the apparent expansion during this period is obtained for our experiments as $\frac{D}{c_{1e}} - \frac{D}{c_1}$ where D is the amount of dye injected, c_{1e} is the concentration at one minute on Gibson and Evans' ex-

ponential extrapolation, and c_1 is the actual concentration at one minute on our log.-log. smoothed plot. It is obvious from the figure that the apparent expansion of the dye distribution volume during the rapid disappearance phase very nearly represents the excess of the dye-injection over the dye-decrement volume. The correlation between these two variables is difficult to explain on the basis

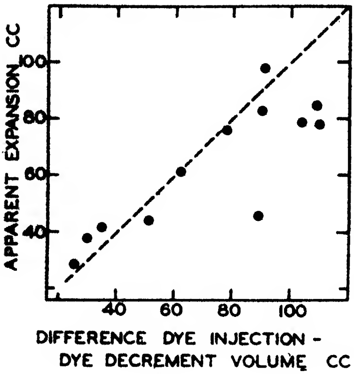

FIG. 2. The ordinates show the apparent expansion of the dye distribution volume during the 'mixing period,' calculated from $\frac{D}{c_{1e}} - \frac{D}{c_1}$, where D is the amount of dye injected, c_{1e} is the concentration at one minute obtained by extrapolating the later exponential slope and c_1 is the actual concentration at one minute read on the log.-log. smoothed plot. The abscissae show the difference between the conventional dye-injection value for plasma volume and the volume obtained by the dye-decrement method. The dashed line is drawn at perfect agreement.

TABLE 2. *Comparative values for dye distribution when injected in a large volume of plasma and a small volume of aqueous solution (saline)*

The values given under the heading *Dye* are the dye distribution volumes obtained by the method of Gibson and Evans with injection of 10 to 20 cc. of conventional dye solution. The values given under *Dyed plasma* are the dye distribution volumes obtained by the same calculation (see text) with the injection of 100 to 150 cc. of dyed plasma (10 cc/kgm.) after correction for the volume of plasma added to the circulation. All volumes are given in cubic centimeters.

DOG NO.	DYE	DYED PLASMA	DYED PLASMA/DYE	EXPERIMENTAL CONDITION
13	461	489	1.059	Dye first, bleeding
14	654	660	1.010	Dyed plasma first, no bleeding
15	532	514	0.966	Dyed plasma first, no bleeding
16	530	502	0.950	Dyed plasma first, bleeding
17	558	554	0.992	Dye first, no bleeding
18	362	379	1.047	Dye first, no bleeding
19	349	327	0.933	Dyed plasma first, bleeding
Average			0.985	

of any systematic error in the dye-decrement method. If, on the other hand, the dye-decrement method yields a more nearly true value for plasma volume, this correlation would be expected. The error in the volume obtained by Gibson and Evans' method should be a function of the dye lost from the plasma compartment during the initial rapid disappearance phase.

Controls on the validity of the dye-decrement method may be obtained by

substituting dyed for undyed plasma in otherwise identical experiments. Plasma volume may be calculated by the dye-decrement formula or by the conventional formula after converting the volume of dyed plasma into dye dosage. The latter calculation yields identical results if the injected volume of plasma is deducted. Table 2 shows the values obtained for plasma volume by the injection of dyed autogenous plasma in such experiments. In all the experiments of the table, the value obtained with dyed plasma agrees within 7% with the value obtained by the conventional dye-injection method. The disagreements are random in direction, with an average for the group of only 1.5% difference.

DISCUSSION. The control studies with dyed plasma injection fail to reveal any systematic error in the dye-decrement method. Circulatory disturbances or errors in the basic assumptions capable of producing a systematic difference between the dye-injection volume and the dye-decrement volume should also systematically influence the apparent distribution volume of injected dyed plasma. In the control studies, however, the distribution volume of dyed plasma is not consistently different from that of dye injected in the usual manner. Since it is inconceivable that the presence or absence of dye in the injected plasma will actually influence the distribution of the plasma within the circulation, the difference between dyed and undyed plasma must mean that dye leaves the former to distribute itself beyond the plasma compartment.

The time-concentration curves are treated in exactly the same manner in these studies in obtaining the dye-injection and the dye-decrement volume. For both calculations an exponential post-injection slope is extrapolated to the time of the injection to obtain the change in optical density after complete mixing and before loss of injected material. The calculations are fundamentally identical, and based on identical assumptions. In both, a constant rate of change is assumed to begin at the time of the injection, with the possibility of masking during an initial mixing period. After undyed plasma injection, such a constant rate of change is achieved within a maximum period of 5 minutes, usually within the first 2 minutes. After dye injection, or the injection of dyed plasma, as long as 20 minutes may be required. The assumption of a constant rate of change thus comes closer to empirical verification with the dye-decrement method. Since the disagreement between the dye-injection and the dye-decrement volume must mean that the common basic assumptions are not equally valid for the two methods, the dye-decrement method would appear to yield a more reliable estimate of plasma volume.

Early dye methods allowed a supposedly minimum time for circulatory mixing, usually of the order of 3 minutes, and sampled as soon as possible thereafter in order to minimize dye lost (9). The empirical agreement between our one-minute dye volumes and our dye-decrement volumes suggests that the former may be useful in obtaining an approximate value for plasma volume. It must be recognized, however, that the agreement may be fortuitous. Mixing of plasma solute is not always complete within one minute, and there is no reason to believe that dye escape from the plasma compartment will be delayed for a full minute after the injection. It is possible that these two factors, with oppo-

site effects on dye concentration, happen to summate in such fashion at one minute as to yield a true value for the plasma volume.

SUMMARY

Plasma volume calculated as the distribution volume of the dye T-1824 by the method of Gibson and Evans was found in barbitalized dogs to be consistently larger than the value found for the distribution volume of subsequently injected undyed plasma. The latter volume (dye-decrement volume) averaged approximately 85% of the dye distribution volume in a series of 12 animals, with extremes of 77 and 93%. When the distribution volume of dye injected in a large volume of plasma was measured in control experiments, it was found to be in good agreement with the volume obtained for small volumes of concentrated dye solution.

When the distribution volume was calculated from the dye concentration observed at one minute on a log.-log. smoothed plot of the rapid disappearance phase, the values were found to be in fairly good agreement, as a rule, with the dye-decrement volumes. When a large amount of dye disappeared from arterial plasma during the rapid disappearance phase, the apparent distribution volume of the dye as obtained by the method of Gibson and Evans was greatly in excess of the distribution volume of undyed plasma. When only a small dye disappearance was observed, the two distribution volumes were more nearly in agreement.

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EFFECT OF PLASMA INJECTION ON DYE AND CELL CONTENT OF ARTERIAL BLOOD

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Received for publication August 21, 1947

If the blood cells are uniformly distributed throughout the blood volume (1), the total volume of cells may be calculated as $C = P \times \frac{c}{p}$, where P is the plasma volume and $\frac{c}{p}$ is the ratio cells: plasma in a sample of blood. When current dye methods are used to obtain the plasma volume, the cell volume calculated by this formula is consistently larger than that obtained by injecting tagged red cells (2, 3). It is not known to what extent this discrepancy is due to overestimation of the plasma volume by the dye method and to what extent it is due to concentration of cells in the more rapidly circulating blood from which the samples are drawn. The studies of Gibson, *et al.* (4) on the difference between the small vessel and the large vessel hematocrit cannot be considered definitive, since no assurance can be given that the tagged plasma protein used for measuring the small vessel plasma volume remains entirely intravascular.

In measuring plasma volume by the dye-decrement method, which is described in detail elsewhere (5), a fairly large volume of undyed plasma is injected into an animal whose circulating plasma contains the dye T-1824. There is a simultaneous decrease in the dye concentration and the cell content of circulating blood. If the distribution of cells throughout the blood volume is sufficiently uniform to permit the total cell volume to be calculated as above, it should be possible to predict the arterial hematocrit reduction which will be observed with a given reduction in plasma dye concentration. In the present study these predicted values are derived and compared with the observed values. In addition, an independent value for the total cell volume is calculated from the hematocrit change on the assumption that the arterial hematocrit bears a constant relationship to the total body hematocrit.

METHODS AND RESULTS. The studies were done on splenectomized dogs, in a steady state of barbital anesthesia. Descriptions are given elsewhere of the preparation and injection of autogenous plasma and cell concentrates and the measurement of dye concentrations and hematocrits (6). All determinations were made on arterial blood. Since arterial hematocrits usually become stable within a maximum of 4 minutes after injection of a large volume of either plasma or cells, hematocrit values were obtained as simple averages of 4 to 12 samples drawn at intervals of 3 to 10 minutes after this initial period. In rare instances progressively declining hematocrits were observed for as long as 10 minutes after

plasma injection. For the sake of uniformity, this unexplained phenomenon was ignored in obtaining the average hematocrit values. To obtain the volume of cells withdrawn in bleeding, the volume drawn was usually multiplied by the average cell: plasma ratio of the preceding period, as this method appeared to have a smaller technical error than that involved in sampling a large volume of blood after it had been drawn and allowed to stand for a time.

In order to prevent expansion of the blood volume, the plasma injections were immediately preceded by rapid withdrawal of equal volumes of blood. With uniform distribution of cells throughout the entire blood volume, the arterial hematocrits before and after the plasma injection should have the following relationships:

$$\frac{H_2}{H_1} = \frac{C_1 - C_e}{B_1} \times \frac{B_1}{C_1}$$

and

$$H_2 = \frac{C_1 - C_e}{C_1} \times H_1 \tag{1}$$

where H_1 and H_2 are the hematocrits before and after the injection stated as the ratio cells: total volume in the samples, C_1 is the initial volume of cells in the animal, C_e is the volume of cells withdrawn in the bleeding and B_1 is the initial, unchanged blood volume. Furthermore, with uniform cell distribution, the total volume of cells in the blood volume is

$$C_1 = \frac{P_1H_1}{1 - H_1} \tag{2}$$

where P_1 is the plasma volume. Since, therefore,

$$\begin{aligned} B_1 &= P_1 + C_1 \\ &= P_1 + \frac{P_1H_1}{1 - H_1} \end{aligned}$$

equation 1 may be written:

$$H_{2e} = \frac{P_1H_1 + C_eH_1 - C_e}{P_1H_1} \times H_1 \tag{3}$$

where H_{2e} is the arterial hematocrit expected after the plasma injection on the basis of the theory which is under examination.

The dye decrement estimate of plasma volume is based on the decrease in plasma dye concentration (or optical density) following injection of undyed autogenous plasma into a dyed animal. The value obtained is

$$P_1 = \frac{R(E_m - E_r)}{E_p - E_m} + P_e \tag{4}$$

where R is the volume of plasma injected, E_m is the optical density of the circulating plasma after the injection, E_p the density before, E_r the density of the un-

dyed injected plasma and P_e the volume of plasma withdrawn in the preceding bleeding. When this value for P_1 is used in equation 3, accordingly, the value obtained for $H_{2\theta}$ becomes a function of the decrease in plasma-dye concentration. The calculation yields the hematocrit which is expected if there is an equivalent decrease in the dye and the cell content of arterial blood.

Table 1 gives the data required for solution of equation 3, in a series of experiments, and shows the hematocrits which were observed as compared with the predicted values. As shown in the table, the observed hematocrit was always

TABLE 1. *Comparison of the expected and the observed arterial hematocrits after injection of undyed plasma in dyed animals*

The symbols in the column headings have the same meaning as in equation 3 in the text. P_1 is the plasma volume as obtained from the decrease in the dye concentration of circulating plasma (dye-decrement method). C_e is the volume of cells withdrawn. H_1 and H_2 are the average arterial hematocrits before and after the plasma injection, stated as the ratio of packed cell-volume to total volume of sample. $H_{2\theta}$ is the hematocrit expected after the injection as calculated from equation 3.

EXP. NO.	P_1	C_e	H_1	H_2	$H_{2\theta}$
	cc.	cc.			
1	363	57.80	0.5780	0.4688	0.5100
2	411	48.75	0.5425	0.4633	0.4885
3	361	56.10	0.5788	0.4928	0.5135
4	570	86.10	0.5374	0.4285	0.4678
5	490	41.36	0.4136	0.3504	0.3632
6	345	50.53	0.5053	0.4148	0.4337
7	296	48.08	0.4808	0.3432	0.3965
8	466	68.50	0.5000	0.4020	0.4265
9	335	48.26	0.4826	0.3900	0.4078
10	385	54.75	0.5475	0.4710	0.4830
11	525	54.90	0.4232	0.3435	0.3627
Average			0.5082	0.4153	0.4412

lower than the predicted value. When the hematocrits were plotted against time following the injection, the entire time plot lay below the predicted level in most cases. The table shows that the average hematocrit reduction following plasma injection is approximately 140% of the reduction expected from the decrease in dye concentration.

Even though these results fail to support the working hypothesis and suggest that the arterial hematocrit is considerably in excess of the total body hematocrit, equation 2 could be solved for the total cell volume if the arterial hematocrit has a constant relationship to the hematocrit of the entire blood volume. If the distribution of cell-poor and cell-rich blood is not changed by the bleeding and plasma injection, and if the total blood volume remains the same, the following equations are valid:

$$C_2 = C_1 - C_e = B_1H_1k - C_e = B_1H_2k$$

where C_1 is the cell volume before the bleeding and plasma injection, C_2 the cell volume after, C_e is the volume of cells withdrawn and replaced with plasma, B_1

is the initial, unchanged blood volume and k is a constant whose value is less than one. Accordingly,

$$B_1 = \frac{C_e}{H_1k - H_2k}$$

and

$$B_1H_1k = \frac{C_e}{H_1k - H_2k} \times H_1k.$$

But B_1H_1k is equal to C_1, which is wanted, and the constant cancels out. Therefore,

$$C_{1h} = \frac{C_e}{H_1 - H_2} \times H_1 \tag{5}$$

where C_{1h} is the initial cell volume, before the bleeding and plasma injection, as obtained from the change in arterial hematocrit. Although no term for plasma volume appears in this equation, the equation is based on the assumption of an unchanged total blood volume, as is equation 4 for plasma volume, and is subject to a similar error if this condition is violated. Aside from this, the determination of plasma volume by equation 4, and of cell volume by equation 5, are totally independent.

The hematocrit for the entire blood volume is obviously

$$H_b = \frac{C_h}{C_h + P_{dd}} = H_a k,$$

if the cell volume C_h as obtained from the hematocrit change is the true cell volume, the plasma volume P_{dd} as obtained by the dye-decrement method is the true plasma volume and H_a is the arterial hematocrit. Under these conditions the ratio between the hematocrit of the entire blood volume and the hematocrit of arterial blood is

$$k = \frac{C_h}{(C_h + P_{dd})H_a} \tag{6}$$

Table 2 gives the values found for cell volume in our experiments by the hematocrit-change method of equation 5, and by two calculations from equation 2, based on the arterial hematocrit and the plasma volume. One of the latter calculations uses the plasma volume as found by the conventional dye-injection method (C_{pdi} in the table), and the other uses the smaller plasma volume found by the dye-decrement method (C_{pdd} in the table). As would be expected from the foregoing data, the value found for cell volume was always largest when it was calculated from equation 2 with plasma volume as obtained by dye injection, and smallest when it was calculated from the hematocrit change. In this small series, the hematocrit-change method gave a value for cell volume which averaged about 61% of the dye injection-arterial hematocrit calculation. When the dye-decrement plasma volume was substituted for the dye-injection volume, the cell volume obtained by equation 2 was reduced to about 84%. If the hematocrit-change method yields a true value for cell volume, therefore, it appears that

in these experiments about half the overestimation in deriving cell volume as C_{pdi} is due to overestimation of the plasma volume, and about half is due to the difference between arterial and total body hematocrit. The last column of the table gives the coefficient required, on the basis of the present assumptions, to obtain the total body hematocrit from the arterial hematocrit (k from equation 6).

TABLE 2. *Comparative values for total cell volume*

Same experiments as in table 1. The column headed C_{pdi} gives the values for total cell volume calculated from equation 2 in the text (plasma volume by cell: plasma ratio) when the plasma volume is obtained by the dye-injection method. The column headed C_{pdd} gives the total cell volumes calculated from equation 2 when the plasma volume is obtained by the dye-decrement method, i.e., P_1 in table 1. The column headed C_h gives the total cell volumes calculated from the change in arterial hematocrit by equation 5. All cell volumes have been corrected for cells drawn in sampling and bleeding. The last column gives the values found for the ratio of total body hematocrit to arterial hematocrit (k) found by equation 6 in the text.

EXP. NO.	C_{pdi}	C_{pdd}	C_h	C_{pdd}/C_{pdi}	C_h/C_{pdd}	C_h/C_{pdi}	k
	cc.	*cc.*	*cc.*				
1	568	494	306	0.869	0.620	0.538	0.795
2	593	485	334	0.818	0.689	0.563	0.828
3	646	495	377	0.767	0.762	0.583	0.882
4	756	663	425	0.878	0.641	0.562	0.794
5	389	345	271	0.887	0.784	0.697	0.861
6	379	353	283	0.931	0.802	0.746	0.894
7	321	273	168	0.850	0.616	0.523	0.753
8	576	466	349	0.809	0.749	0.606	0.856
9	355	313	252	0.882	0.806	0.709	0.888
10	619	466	392	0.754	0.840	0.634	0.923
11	503	385	292	0.766	0.758	0.581	0.844
Average				0.838	0.733	0.613	0.847

If the basic assumptions for the hematocrit-change calculation of cell volume are valid, it should be possible to estimate the cell volume from the hematocrit increase following injection of cell-concentrates as well as from the decrease following plasma injection. If the drawn blood is replaced with an equal volume of cell-rich blood, equation 5 becomes

$$C_{1h} = \frac{C_r - C_c}{H_2 - H_1} \times H_1 \tag{7}$$

where C_r is the volume of cells injected, and the other terms have the same meaning as before. Table 3 shows experiments in which the hematocrit-change calculation for cell volume was made from the hematocrit increase following cell-concentrate injections as well as from the hematocrit decrease following plasma injection. If a correction is made for the cells actually withdrawn or added, the table shows fairly good agreement between the cell volume as determined in

an animal by injecting plasma at one time and cells at another. The average ratio $C_h:C_{pdi}$ for all the cell injections happens to be identical with the average ratio for the plasma injections, with a value of 0.767. That is to say, in this group of animals, the hematocrit change-method yields a value for cell volume which averages 76.7% of the conventional plasma volume-arterial hematocrit value, whether the hematocrit is increased or decreased in applying the method.

TABLE 3

In the column headed 'Injection', P indicates injection of plasma and C injection of cells. The column headings C_{pdi} and C_h have the same meaning as in table 2. The last 3 columns give the volume of cells added or withdrawn during the interval between determinations, and the circulating cell volumes corrected accordingly.

EXP. NO.	INJECTION	C_{pdi}	C_h	C_h/C_{pdi}	CUMULATIVE CHANGE CELL VOLUME	C_{pdi} CORRECTED	C_h CORRECTED
		cc.	cc.		cc.	cc.	cc.
19	C	306	272	0.888			
20	P	378	281	0.743	0	378	281
	C	337	215	0.638	−54	391	269
	P	308	232	0.752	−13	321	245
21	P	696	598	0.860	0	696	598
	C	627	481	0.767	−104	731	585
	P	613	524	0.853	−60	673	584
22	C	570	505	0.886	0	570	505
	P	628	568	0.904	+60	568	508
23	C	571	355	0.621	0	571	355
	P	660	400	0.606	+65	595	335
24	C	247	206	0.835	0	247	206
	P	369	240	0.650	+55	314	195
25	C	373	273	0.732			

DISCUSSION. The excessive fall in the arterial hematocrit with cell withdrawal in these studies is in agreement with previous data obtained under less well-controlled conditions (7–10). Hahn and Bale (11) plotted the declining venous hematocrit against the declining total cell volume as obtained by radio-iron in chronic bleeding experiments. They found that although the two variables were linearly related, the rate of hematocrit decrease was less than that of the total cell volume. Since no attempt was made to ensure the maintenance of a constant blood volume in their experiments, and in the absence of published data on plasma volume, it appears likely that this paradoxical finding was due to incomplete restitution of the plasma loss.

In the cell-washout studies of Lawson *et al.* (10) the arterial hematocrit was found to decline at a constant exponential rate to the death of the animal when fixed volumes of blood were drawn at regular intervals and replaced with equal volumes of isotonic gelatin solution. The total cell volume was calculated as the extrapolated total washout from the formula

$$Y = \frac{y}{1 - r},$$

where Y is the total washout, y the yield of cells on the first bleeding and r the constant ratio for the series of diminishing yields. It is obvious that the hematocrit-change calculation of cell volume in the present study is essentially a one-step washout calculation. If a constant volume of blood is drawn in the bleedings of the washout, the successive cell yields become simple functions of the hematocrits in the drawn blood. If the yields are $y, yr, yr^2 \cdots yr^{(n-1)}$, the value of r may thus be obtained as the ratio of any two successive hematocrits. Accordingly, the washout equation may be written

$$Y = \frac{y}{1 - \dfrac{H_2}{H_1}}$$

$$= \frac{y}{H_1 - H_2} \times H_1,$$

which is identical with equation 5 in the present study for cell volume by the hematocrit-change calculation. The validity of the present method for cell volume is therefore the same, in theory, as that of the previously reported washout method. It is technically superior, in that autogenous plasma is used and the interval between the two hematocrit readings is indefinitely long, so that mixing of the replacement fluid with circulating blood should be quite complete. The hematocrit-change method has been employed in a total of 33 instances in the present studies, including experiments with dyed plasma injection not reported. The cell volume found by this method averaged 70.7% of that calculated from the arterial hematocrit and the dye-injection value for plasma volume. The somewhat higher percentage found in the previous study (average about 84%) may have been due to the use of a foreign colloidal solution, gelatin, for the washout, or it may have been due to the method used in calculating plasma volume which yields a somewhat smaller figure than that obtained for the dye-injection plasma volume in the present study.

SUMMARY

Equivalent reductions in the dye and the cell content of arterial blood, following the injection of undyed plasma, into dyed dogs, were calculated as theoretical values on the assumption of an homogeneous distribution of the total cell mass throughout the entire blood volume. Plasma volume for these calculations was obtained *a*) by a conventional dye injection method and *b*) by the dye-decrement method. The cell mass was calculated from the arterial hematocrit and the plasma volume. Even when the smaller value obtained for plasma volume by the dye-decrement method was employed, the hematocrit reductions observed in barbitalized, splenectomized dogs following plasma injection were consistently excessive, with an average of approximately 140% of the expected reductions.

An independent value for the total volume of cells was obtained from the change in arterial hematocrit following injection of either plasma or cell concentrates. The cell volume obtained by this method averaged about 71% of the

volume calculated from the arterial hematocrit and the dye-injection plasma volume and about 84% of the volume calculated from the arterial hematocrit and the dye-decrement plasma volume. The data suggest, accordingly, that the conventional calculation of total cell volume from the plasma volume and the arterial hematocrit yields a false high value; and that about half the error is due to overestimation of the plasma volume by the dye-injection method, about half to overestimation of the total body hematocrit from the hematocrit of arterial blood.

The total body hematocrit, as obtained from the dye-decrement value for plasma volume and the hematocrit-change method for cell volume, was found to average about 85% of the arterial hematocrit, with extreme values of approximately 75 and 92% in a small series.

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RENAL TUBULAR REABSORPTION OF INORGANIC SULFATE IN THE NORMAL DOG[1]

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Received for publication August 26, 1947

Inorganic sulfate is formed during the metabolism of the sulfur-containing amino acids. Although its exact functions are not well understood, the body nevertheless maintains a basic store of circulating sulfate at all times. Its plasma level, although low, is fairly constant, being maintained in the normal dog within limits of 1.2 and 1.8 millimols per liter. The maintenance of plasma concentration within these limits is brought about by renal mechanisms, an understanding of which is requisite for a complete picture of the renal regulation of electrolyte balance. Since the sulfate ion has been shown to be completely filterable through the glomerular membrane (5, 4), the stabilization of its plasma concentration depends, fundamentally, upon its tubular reabsorption, for upon the characteristics of this process depends the balance between its conservation and elimination by the kidneys.

The urinary excretion of sulfate was studied in man by Hayman (5), who found that following the intravenous administration of sodium sulfate, the urine plasma concentration ratio of sulfate approached but never quite reached that of creatinine. This was cited as evidence that some sulfate was reabsorbed by the tubules. In the dog, Goudsmit, Power and Bollman (4) found that at elevated concentrations of sulfate in the plasma, the sulfate clearance approaches, as an asymptote, the creatinine clearance. They too inferred that some sulfate was reabsorbed by the tubules and although they did not analyze their data to prove their point, they indicated that the rate of reabsorption of sulfate was limited at high plasma-sulfate concentrations. More recently the problem was re-investigated in the dog by Schwartz, Smith and Winkler (15), who reached the opposite conclusion, namely, that the capacity to reabsorb sulfate increases directly with the quantity filtered through the glomeruli. Their experiments were complicated by wide variations in urine flow, rapid changes in serum sulfate concentration and progressively declining rates of glomerular filtration. These factors render their conclusions open to question.

The experiments presented below were performed in order to study the characteristics of the renal tubular reabsorption of inorganic sulfate in the normal dog. They indicate that the capacity to reabsorb sulfate is limited, and under normal conditions is unaffected by changes in the rate of reabsorption of other electrolytes.

METHODS. Experiments were performed on two female mongrel dogs trained to lie with loose restraint on a comfortable animal table. Urine collections were

[1] This study was aided by grants from the John and Mary R. Markle Foundation and the United States Public Health Service.

made with an indwelling catheter. The collection periods were 10 minutes long, and when the urine flow was less than 10 ml. per minute, the bladder was washed with distilled water and the washings added to the original urine. A blood sample was drawn from the jugular vein at the midpoint of each collection period, oxalated and centrifuged for plasma analyses. Infusions were administered continuously by saphenous vein at rates of 3, 4 and 10 ml. per minute. Each infusion was allowed to run for 20 minutes before the experimental periods were begun to allow plasma concentrations to become nearly constant. The infusion mixtures, which were made isotonic with glucose, contained creatinine for the measurement of glomerular filtration rate and varying amounts of sodium sulfate according to the plasma concentration of sulfate desired. Water was given in amounts of 50 ml. per kilo of body weight approximately one hour before the experiment to insure adequate hydration. Creatinine was determined on iron

TABLE 1. *Experiment on a normal dog showing relation between amount of inorganic sulfate filtered through the glomeruli and amounts reabsorbed by the tubules and excreted in the urine*

TOTAL CONCURRENT TIME	URINE FLOW	GLOMERULAR FILTRATION RATE	INORGANIC SULFATE				
			Plasma	Urine	Filtered	Excreted	Reabsorbed
Experiment 5; Dog 1							
min.	*ml./min.*	*ml/min.*	*mM/L*	*mM/L*	*mM/min.*	*mM/min.*	*mM/min.*
80 *Infuse 0.0% Na_2SO_4, 3 ml. per minute*							
100–110	2.8	59.2	1.62	0.638	0.096	0.002	0.094
110–120	3.8	62.0	1.62	0.386	0.100	0.001	0.099
122 *Infuse 2.0% Na_2SO_4, 3 ml. per minute*							
140–150	5.6	59.6	2.92	9.46	0.174	0.053	0.121
150–160	6.3	59.9	3.80	11.5	0.228	0.104	0.124
162 *Infuse 3.7% Na_2SO_4, 3 ml. per minute*							
190–200	6.8	70.6	8.28	66.7	0.585	0.454	0.131
200–210	6.7	71.1	9.50	81.5	0.675	0.546	0.129

filtrates of plasma (17) and diluted urines by the method of Folin and Wu (2). Sulfate was precipitated by benzidine from trichloracetic acid filtrates of plasma and urine and determined by alkalimetric titration according to the method of Power and Wakefield (13).[2] Phosphate was determined on plasma and urine by the method of Fiske and Subbarow (1) as modified by Pitts (8). Chlorides were analyzed in plasma and urine by the Volhard titration after open Carius digestion as described by Van Slyke (7). All colorimetric analyses were made with an Evelyn photoelectric colorimeter.

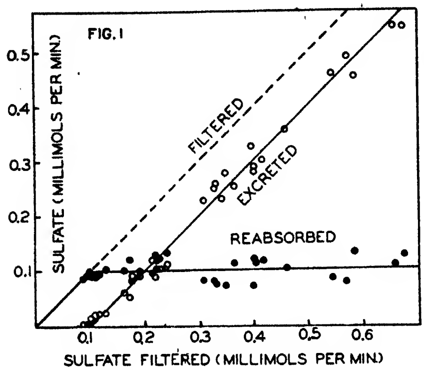
RESULTS. *Reabsorption and excretion of inorganic sulfate as a function of plasma sulfate concentration.* The relation between the quantities of sulfate filtered, excreted and reabsorbed and plasma sulfate concentration is illustrated

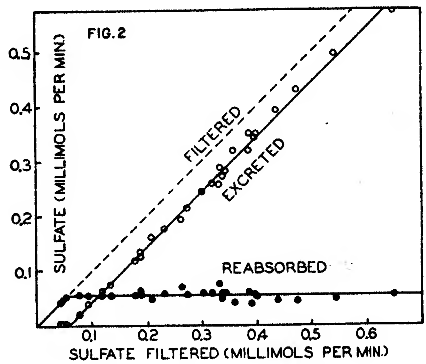
[2] In those experiments in which sulfate and phosphate were simultaneously administered, phosphate was not removed prior to sulfate determination. Preliminary studies had shown that the recovery of sulfate was 100% in the presence of appreciable quantities of phosphate.

in the experiment of table 1. It is evident from the second two columns that rate of glomerular filtration and urine flow were within a physiologic range throughout the experiment. For the 12 hours preceding the experiment food was withheld, and as a result plasma sulfate concentration was at the control level of 1.62 millimols per liter. As a consequence of this low plasma concentration, the quantity of sulfate filtered was small, amounting to only 0.100 millimol per minute. Of this amount filtered, essentially all was reabsorbed, only traces appearing in the urine. Plasma sulfate was then progressively elevated by the intravenous infusion of sodium sulfate. It is seen that in the next four periods the plasma sulfate concentration exceeded the normal value of 1.62 millimols per liter, ranging from 2.9 to 9.5 millimols per liter in the last period. Reference to the last column shows that during these periods the quantity of sulfate reabsorbed remained nearly constant at 0.12 to 0.13 millimols per minute. All of the sulfate filtered over and above the limited quantity reabsorbed was excreted in the urine. This experiment indicates that there exists a threshold above which the rate of tubular reabsorption of sulfate becomes constant, whereas the excess filtered is quantitatively excreted. In figures 1 and 2 the quantities of sulfate reabsorbed and excreted are related to the quantity filtered, i.e., the quantity presented to the tubules in the glomerular filtrate. Figure 1 represents the grouped data of 31 similar clearance periods on *dog 1*, and figure 2, 29 clearance periods on *dog 2*. It is seen in figure 1 that when the quantity of sulfate filtered was below 0.1 millimol per minute, all filtered was reabsorbed and essentially none appeared in the urine. But as the quantity filtered exceeded 0.1 millimol per minute, the quantity reabsorbed remained almost constant at a value that averaged 0.1 millimol per minute. The excess that was filtered over and above this limited amount reabsorbed was excreted in the urine. A limitation of reabsorptive capacity is evident from these data. In the data of figure 2 it is apparent that tubular reabsorption of sulfate was essentially complete when the quantity filtered remained below 0.05 millimol per minute. Above this amount, a constant rate of reabsorption was rapidly attained which amounted to 0.05 millimol per minute. It is evident that this dog reabsorbed on an average 0.05 millimol per minute less than the preceding one. However, a limitation of reabsorptive capacity is here equally apparent. Such quantitative differences as are evident in the reabsorptive capacities of these two dogs are no doubt expressions of individual variations in renal function. The limitation in the rate of tubular reabsorption of sulfate is reproducible in any one individual from day to day and constant over a wide range of plasma sulfate concentrations.

The results of 64 clearance periods on the two dogs are presented in figure 3 in which the sulfate/creatinine clearance ratios are plotted against plasma-sulfate concentration. It is apparent that the sulfate clearance rapidly increases as plasma-sulfate concentration is elevated above normal. The glomerular clearance represented by the dotted line is approached as the asymptote and apparently reached at infinitely high concentrations of sulfate in the plasma. These findings are in agreement with those of Goudsmit, Power and Bollman (4).

The instability of the sulfate reabsorptive mechanism during periods of increased chloride excretion. It has been shown that the tubular reabsorptive mechanisms for chloride and bicarbonate ions are interrelated (9). The capacity to reabsorb bicarbonate is reduced by an increased rate of chloride reabsorption, and vice versa an increased rate of bicarbonate reabsorption reduces the capacity of the tubules to reabsorb chloride. Previous observers (15) have noticed that the





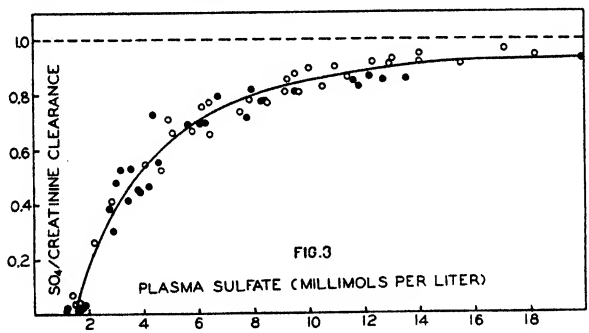


FIG. 1. Quantities of inorganic sulfate excreted and reabsorbed as a function of the quantity filtered. *Dog 1.* Black dots, reabsorbed; circles, excreted.

FIG. 2. Quantities of inorganic sulfate excreted and reabsorbed as a function of the quantity filtered. *Dog 2.* Black dots, reabsorbed; circles, excreted.

FIG. 3. Sulfate: creatinine clearance ratios as a function of plasma-sulfate concentration. Data were derived from 64 clearance periods on two dogs. Circles, experiments on *dog 1;* dots, experiments on *dog 2.*

simultaneous administration of sodium chloride and sodium sulfate enhances the excretion of sulfate. This observation suggests a possible tubular interaction between sulfate and chloride similar to that for chloride and bicarbonate. Experiment 22, table 2, presents an experiment designed to investigate this possibility. By the administration of sodium sulfate, plasma sulfate concentration was maintained at a constant level, sufficiently high to saturate the sulfate reabsorptive mechanism. Sodium chloride was then infused in a 5%

solution in order to elevate plasma chloride concentration. Note that this quantity increased from normal levels of 92.0 millimols per liter in the control periods to 140.0 millimols per liter in the last period. Reference to the last column shows that there was a significant increase of about 27% in the reabsorption of chloride in the second three periods. This increase in chloride reabsorption was the result of the increase in glomerular filtration rate (6) that occurred as a consequence of the infusion of hypertonic sodium chloride. The significant result of this experiment was the decrease of approximately 50% in

TABLE 2. *Experiments illustrating the interrelationships between the tubular reabsorptive mechanisms for chloride and inorganic sulfate in the dog*

TOTAL CONCURRENT TIME	URINE FLOW	GLOMERULAR FILTRATION RATE	PLASMA		URINE		SULFATE			CHLORIDE		
			Sulfate	Chloride	Sulfate	Chloride	Filtered	Excreted	Reabsorbed	Filtered	Excreted	Reabsorbed
Experiment 22; Dog 2												
min.	*ml./min.*	*ml./min.*	*mM./L*	*mM./L*	*mM./L*	*mM/L.*	*mM/min.*	*mM/min.*	*mM/min.*	*mM/min.*	*mM/min.*	*mM/min.*
60 *Infuse 0.5% Na_2SO_4, 0.0% NaCl; 10 ml/min.*												
80–90	5.2	36.7	3.85	94.0	18.4	2.80	.141	.096	.045	3.45	.014	3.44
90–100	5.5	36.3	3.95	92.0	17.9	2.00	.143	.098	.045	3.34	.011	3.33
100–110	6.4	37.4	4.02	92.2	16.1	3.60	.151	.103	.048	3.45	.023	3.43
113 *Infuse 0.5% Na_2SO_4, 5.0% NaCl; 10 ml/min.*												
135–145	19.5	46.3	3.72	120.6	7.35	83.4	.172	.143	.029	5.60	1.62	3.98
145–155	16.9	49.2	3.62	129.8	8.85	122.4	.178	.150	.028	6.40	2.06	4.34
155–165	16.7	48.5	3.75	140.0	9.30	151.8	.182	.155	.027	6.79	2.54	4.25
Experiment 23; Dog 2												
60 *Infuse 0.5% Na_2SO_4, 0.0% NaCl; 10 ml/min.*												
80–95	5.5	38.7	2.87	97.0	14.3	5.20	.111	.079	.032	3.75	.028	3.72
100–110	7.1	38.8	2.98	95.4	12.5	5.20	.116	.089	.027	3.70	.037	3.66
110–120	7.9	37.2	3.37	96.6	11.5	9.20	.125	.091	.034	3.60	.073	3.53
125 *Fed meat*												
375 *Infuse 0.5% Na_2SO_4, 0.0% NaCl; 10 ml/min.*												
395–405	10.3	44.7	3.12	101.4	11.5	11.8	.140	.118	.032	4.51	.121	4.39
405–415	9.4	43.1	3.30	100.8	11.4	7.20	.142	.107	.035	4.35	.068	4.28
415–425	7.5	39.7	3.37	100.2	13.3	1.20	.134	.100	.034	3.97	.090	3.88

the capacity of the tubules to reabsorb sulfate that occurred in the last three periods. In view of the conditions of this experiment, there are two possible explanations for this finding. First, the depression of sulfate reabsorption could have occurred as a specific effect of the increase in the tubular reabsorption of chloride, i.e., that the chloride and sulfate ions could have competed for a common reabsorptive mechanism. Second, the depression in sulfate reabsorption could have occurred as a non-specific osmotic effect consequent upon the appearance of large quantities of sodium and chloride ions in the glomerular filtrate. If one were able to effect an increase in the rate of reabsorption of chloride without a concomitant increase in chloride excretion, it would be possible to differentiate

these possibilities. Accordingly, experiment 23 in table 2 was done. Plasma chloride remained normal throughout. The rate of reabsorption of chloride was increased by increasing the rate of glomerular filtration through the feeding of meat. Plasma sulfate was elevated to levels comparable with experiment 22 and three control periods were taken. Two pounds of lean beef were fed and four hours later three identical periods were run. The increase in the rate of glomerular filtration that occurred as a result of this procedure was comparable to that which occurred in the previous experiment. In like manner, an increase of 21% in the rate of reabsorption of chloride compares favorably with the 27% increase in this function in the previous experiment. It is striking, however, that in experiment 23 the reabsorption of sulfate remained remarkably constant during all six periods, in spite of the increased rate of chloride reabsorption. The difference between the two experiments lies in the fact that in the second one there was an increase in the rate of reabsorption of chloride without a concomitant

TABLE 3. *An experiment on a normal dog that illustrates the independence of the tubular mechanisms for the reabsorption of inorganic sulfate and inorganic phosphate*

TOTAL CONCURRENT TIME	URINE FLOW	GLOMERULAR FILTRATION RATE	PLASMA		URINE		SULFATE			PHOSPHATE		
			Sulfate	Phosphate	Sulfate	Phosphate	Filtered	Excreted	Reabsorbed	Filtered	Excreted	Reabsorbed
Experiment 17; Dog 1												
min.	*ml/min.*	*ml/min.*	*mM/L*	*mM/L*	*mM/L*	*mM/L*	*mM/min.*	*mM/min.*	*mM/min.*	*mM/min.*	*mM/min.*	*mM/min.*
50 *Infuse 0.0% Na_2SO_4, phosphate 0.000M, creatinine 0.62%; at 4.0 ml. per minute*												
110–120	5.8	70.5	1.25	.748	.903	0.00	.088	.005	.083	.053	.000	.053
120–130	5.5	64.5	1.24	.780	.553	0.00	.080	.003	.077	.050	.000	.050
130–140	5.7	64.8	1.21	.598	.517	0.00	.078	.003	.075	.039	.000	.039
155 *Infuse 0.0% Na_2SO_4, phosphate 0.034M, Creatinine 0.62%; at 4.0 ml. per minute*												
175–185	10.2	78.2	1.13	1.78	1.22	3.77	.088	.012	.076	.139	.038	.101
185–195	9.6	76.2	1.10	1.98	.935	4.63	.084	.009	.075	.151	.044	.106
195–205	8.5	76.6	1.10	2.15	.745	5.84	.080	.006	.074	.164	.049	.115

change in the rate of chloride excretion. Thus this experiment differentiates the explanations for the results of experiment 23 and indicates that the chloride and sulfate ions do not compete for a common reabsorptive mechanism. The depression in the capacity of the tubules to reabsorb sulfate in experiment 22 must be explained as a non-specific effect of the chloride ion. The exact nature of this action is not clear; however, a theoretical discussion will be presented below.

Independence of the reabsorptive mechanisms for sulfate and phosphate. In table 3 an experiment is presented which illustrates the independence of the mechanisms for the tubular reabsorption of inorganic sulfate and inorganic phosphate. Plasma-sulfate concentration was normal and fairly constant throughout. After three control periods phosphate was infused in amounts sufficient to cause a marked elevation in phosphate reabsorption. It is seen from column 10 that the rate of tubular reabsorption of sulfate remained remarkably constant throughout.

These results demonstrate that the reabsorptive mechanisms for sulfate and phosphate are separate and in no way interrelated.

DISCUSSION. From the data presented in this paper it is evident that there is a plasma threshold for sulfate, below which the kidney salvages essentially all of the sulfate filtered and allows only traces to appear in the urine. With but slight elevations in plasma sulfate concentrations, or even with increases in filtration rate, the plasma threshold is rapidly exceeded, a maximal reabsorptive capacity for sulfate is attained, and any further sulfate that appears in the glomerular filtrate is quantitatively excreted in the urine. These characteristics of the renal mechanisms that handle sulfate are apparent from the graphs in figures 1 and 2. The properties of the sulfate reabsorptive mechanism fit it for its function of stabilizing plasma-sulfate concentration. The reabsorption of a constant amount of this ion assures the conservation of a basic store of circulating sulfate at all times. The rapid elimination of any excess tends to prevent undue expansions of sulfate in the body fluids. This latter characteristic is especially important for the bodily economy. For since the sulfate ion acts as a strong acid, it requires its full complement of base, and any increase in its concentration in the extracellular fluid brings about a depletion of the alkali reserve with serious derangement of the acid-base balance of the body.

Under normal conditions the capacity of the renal tubules to reabsorb sulfate is limited and independent of changes in glomerular filtration and the rates of reabsorption of other ions. These findings signify that inorganic sulfate is handled in a specific fashion different from the other electrolytes by an active type of tubular mechanism. The sulfate reabsorptive mechanism thus exhibits characteristics similar to those that handle glucose (16), amino acids (10, 11), inorganic phosphate (12) and ascorbic acid (3,14), in which a limitation of reabsorptive capacity is felt to be dependent upon limited quantities of some tubular components utilized in the reabsorptive processes. The depression of sulfate reabsorption that occurs when chloride ion is in high concentration in the tubular urine might be explained in the following way. An increase in the total ionic strength of the tubular urine might make so steep the osmotic gradient against which sulfate must be reabsorbed that the tubular cells are no longer able to deliver sufficient energy to meet the increased osmotic demands. Such an explanation supposes that there exists in the reabsorption of sulfate an 'osmotic ceiling' against which the tubules are unable to work. Thus the capacity of the tubules to reabsorb sulfate would be limited by the same type of osmotic ceiling that limits the reabsorption of water. Any explanation is at present pure speculation, and the solution of the problem must await further metabolic and thermodynamic studies on the tubular cell.

SUMMARY

1. The renal tubular reabsorption of inorganic sulfate has been studied in 23 experiments on two dogs over a range of plasma-sulfate concentrations of 1.2 to 20.0 millimols per liter.

2. It has been observed that sulfate is reabsorbed by an active type of mech-

anism which exhibits a limitation of reabsorptive capacity. Under conditions of normal plasma sulfate and normal rates of glomerular filtration, there is quantitative reabsorption of all sulfate in the glomerular filtrate. With any slight increase in the tubular load, the reabsorptive capacity is rapidly exceeded and the excess is excreted in the urine. Thus the renal threshold for sulfate is very sharp.

3. Under normal conditions the reabsorptive mechanism for sulfate is independent of those of chloride and phosphate.

4. Associated with the infusion of hypertonic sodium chloride there is a marked decrease in the capacity to reabsorb sulfate. This is felt to be a non-specific osmotic effect consequent upon the appearance of large quantities of sodium and chloride ions in the glomerular filtrate. A theoretical discussion of this effect has been presented.

The author wishes to express his thanks to Dr. Robert F. Pitts for his many helpful suggestions, and to Mr. Raymond Cottet for his help in the performance of the experiments.

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PERIPHERAL VISUAL ACUITY OF 55 SUBJECTS UNDER CONDITIONS OF FLASH PRESENTATION[1]

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Received for publication October 2, 1947

Peripheral visual acuity in groups of subjects selected at random has been measured by the writer under various experimental conditions including photopic illumination (1), scotopic illumination (2) and moving test objects (3). A 25-hour course of training by controlled practice yielded test scores obtained by the same technique before, during and after the training (4, 5). Included in this study were acuity scores obtained by a technique in which a shutter timed at $\frac{1}{5}$ second was interposed in the line of peripheral vision. Both before and after training these scores showed weaker acuity than was observed in tests using a technique which presented the test object for an unlimited exposure time. This suggested a systematic investigation of the effects of limitation of stimulus exposure time on peripheral form acuity. The results of such an investigation are reported in this paper.

APPARATUS. All tests were made in a black cloth booth which extended beyond the limits of the subject's visual field. A 25 cm. perimeter illuminated by a 60 w. Mazda daylight bulb was used. The brightness of the white background surrounding the test object was 13.7–14.0 millilamberts. Photographically printed Landolt circles with breaks of 10, 9, 8, 7, 6, 5, 4, 3, $2\frac{1}{2}$, 2, $1\frac{1}{2}$, 1, $\frac{1}{2}$, and $\frac{1}{4}$ mm. were the test objects. The contrast between the black test object and the white background was 97%. An Ilex #4 shutter was interposed in the line of peripheral vision so that the test object was exposed only when the shutter was open. The test objects were interchangeable and when mounted could be rotated around their own center on an axis passing along the line of peripheral vision to the subject's eye. The mechanism of rotation was so regulated that, at the moment of presentation, the break in the circle pointed either up, down, right or left.

METHODS. The peripheral visual acuity of 55 subjects selected at random was measured monocularly on the horizontal meridian in both eyes, the left eye always being tested first. Angular deviations from the line of central vision were 20°, 30°, 40°, 50° and 60°. The shutter was set for exposures of 1, $\frac{1}{5}$, $\frac{1}{25}$ and $\frac{1}{100}$ sec. progressively in that order, a measure for all 5 angular deviations, in the order named, being obtained with each timing of the shutter. This procedure resulted in 20 scores for each eye. During testing the subject tripped the shutter by cable release at a signal from the operator, who had previously set the test

[1] The work described in this paper was done under a contract between the Office of Naval Research and The Johns Hopkins University (Contract N6onr-243, Task Order III, Johns Hopkins University).

object in one of the 4 possible positions mentioned above. The subject then signaled the position he believed the test object to be in. On repeated presentations of any size test object 4 consecutive correct answers before the second wrong one constituted the required success criterion. The subject's score was the midpoint between the size (in mm.) of the break in the smallest circle with which the success criterion was attained and the next smallest circle (on which the subject failed).

Results. The mean scores of the group of 55 subjects are presented in table 1. The mean acuity becomes weaker (numerically larger score) as the angular deviation at which the measure was obtained increases. A decrease in acuity occurs as stimulus exposure time decreases. The mean scores of table 1 are subject to certain limitations. The 20° points are likely to be unreliable because

Table 1. *Simple form acuity in the retinal periphery under conditions of flash presentation in 55 subjects: Means are expressed in mm. in break of Landolt circles*

EXP. TIME		FIRST EYE (LEFT)						SECOND EYE (RIGHT)					
		Angular Deviation					Sum Left	Angular Deviation					Sum Right
		20°	30°	40°	50°	60°		20°	30°	40°	50°	60°	
sec.													
1	Number of failures	*1*	*0*	*0*	*3*	*8*		*0*	*0*	*0*	*1*	*18*	
	Mean	2.4	2.1	2.9	4.2	6.3	17.9	1.7	2.2	3.1	4.4	7.0	18.4
$\frac{1}{5}$	Number of failures	*1*	*1*	*4*	*13*	*36*		*0*	*0*	*1*	*17*	*36*	
	Mean	2.9	3.2	4.7	6.8	9.2	26.8	2.5	2.9	4.1	7.2	9.3	26.0
$\frac{1}{25}$	Number of failures	*0*	*1*	*4*	*18*	*43*		*0*	*1*	*4*	*23*	*44*	
	Mean	3.4	3.7	5.4	7.6	9.9	30.0	3.0	3.8	5.4	8.2	9.7	30.1
$\frac{1}{100}$	Number of failures	*1*	*2*	*9*	*34*	*52*		*0*	*0*	*9*	*33*	*46*	
	Mean	4.1	4.7	6.8	9.1	10.2	34.9	3.6	4.6	7.0	9.1	10.1	34.4
	Sum of means	12.8	13.7	19.8	27.7	35.6	109.6 Total	10.8	13.5	19.6	28.9	36.1	108.9 Total

of the close proximity of the blind spot. Since the Landolt circle is rotated the break may fall on the blind spot. This was noticeable at 20° when the break pointed toward the line of central vision (right in the left eye; left in the right eye), and was nearer the line of central vision by one half the diameter of the circle. Difficulty on the first measure obtained (20°, 1 sec.) was noticeable especially on this position. This, coupled with the newness of the technique, caused a depression of the mean acuity obtained at this point. However, subjects apparently learned to compensate for this difficulty quite easily since the remainder of the 20° points in that eye and the comparable 20° point in the second eye tested (right) did not measure as poorly. Another feature complicating the 20° measures is the possible existence of poor central vision in the eye being tested. This is believed to have little or no effect on the acuity of areas 30° or more from the

line of central vision, but at 20° probably operates with noticeable effect. Therefore some alteration of the scores is to be expected at 20°. Further limitation of the mean scores here presented is traceable to the fact that a 10-mm. circle is the largest used. When failure occurred on size #10 a score was recorded, representing success on a hypothetical size 11. This number (10.4) is doubtless too small, especially when a large number of the group of subjects fail #10. Under circumstances where the acuity is weak, as in areas far removed from the line of central vision and when a short exposure time is used, the mean acuity recorded in table 1 is better (numerically too small) than it actually is. The number of failures on size #10 is recorded in the appropriate boxes in table 1.

The various subtotal scores presented in table 1 point up the differences caused by angular deviation and time of exposure already noted in the individual mean acuities. These subtotals are less susceptible to distortion by the totals for the 20° points. The decreased exposure times caused the greatest numerical loss between 1 and $\frac{1}{5}$ second in both eyes. Less change was observed between $\frac{1}{5}$ and $\frac{1}{25}$ sec., with a slightly greater decrease between $\frac{1}{25}$ and $\frac{1}{100}$ sec. in both eyes. Acuity losses were greatest in the 40°–60° interval of angular deviation from the line of central vision in both eyes with the least change occurring between 20° and 30°. Are these differences between the various subtotal scores true differences due to the specified changes in the experimental situation or are they due to chance alone? This question has been investigated by calculation of the critical ratio between the subtotal scores of consecutively measured angular deviations (20°, 30°, 40°, 50°, and 60°) and consecutively measured exposure times (1, $\frac{1}{5}$, $\frac{1}{25}$, and $\frac{1}{100}$ sec.) by customary formulae (6, pp. 58–61). In the left eye all critical ratios except that between 20° and 30° were higher than 5, indicating, with a probability of 0.99+, that the observed difference was due to controlled differences in the experimental situation. The critical ratio between the 20° and 30° subtotals in the left eye was 1.2, yielding a similar probability of 0.89, but this figure is too low to be acceptable. In the right eye the critical ratio of the same subtotal scores was 5 or above in all cases, yielding a probability of 0.99+, which rules out chance determination of the differences. Between 20° and 30° in this eye the critical ratio was 5.0. The difference between the critical ratios in the two eyes reflects the initial difficulty experienced by the subjects at 20° points because of the presence of the blind spot, which difficulty had been compensated for by the time the second (right) eye was tested. In this connection it it notable that the difficulty with 20° points is found chiefly in the first point tested, 20° at 1 second exposure. It appears from the above evidence that both a 10° increase in the angular deviation of the test object from the line of central vision and the specified decreases in the exposure times cause significant differences in peripheral visual acuity. This conclusion is further supported by an analysis of variance into 4 components by the method recommended by Lindquist (7, chap. 5). This type of analysis extracts the variance of the data under four headings: *a*) angular deviation variance, *b*) exposure time variance, *c*) remainder variance and *d*) variance of scores within each cell. The ratios between *a*) and *c*), and *b*) and *c*) were much

greater than that required for significance at the 1% level of confidence. The ratio between *c*) and *d*) was too great to be due to chance. This discrepancy is interpreted to be due to lack of 'parallelism' in the curves, which in turn may be chiefly traceable to the poor 20° points and the running off the scale of measurement on difficult points. This lack of 'parallelism' is illustrated in figure 1 in which the curves are plotted. The foregoing statements hold true for each eye.

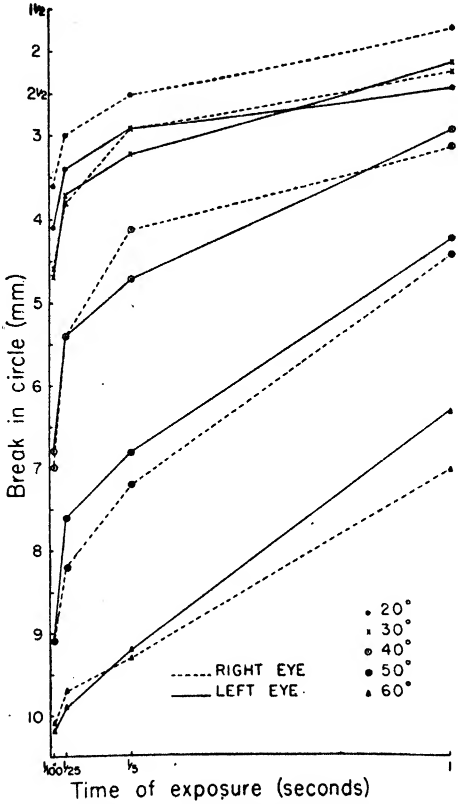

FIG. 1. MEAN PERIPHERAL VISUAL ACUITY SCORES IN 55 SUBJECTS. The acuity is represented in terms of the smallest Landolt circle positionally identified.

Pearson product-moment correlations were made on various of the subtotal scores. Eye-to-eye correlations of subtotal scores of each angular deviation and each exposure time yielded coefficients of from 0.48 to 0.73 without any notable features. The eye-to-eye correlation of the total score for each eye was 0.70 which, when corrected by the Spearman-Brown formula to 0.82, may be interpreted as the reliability of the entire test. Subtotal scores, consisting of the total of all scores in both eyes at each angular deviation and each exposure time,

when correlated with the total score for both eyes revealed the 20° points to be the poorest ($r = 0.53$) with the 60° points ($r = 0.78$) and the 1 sec. points ($r = 0.82$) lower than the remainder of the coefficients which averaged about 0.90. The inter-correlations of the various subtotal scores for angular deviations and exposure times within each eye which were obtained during the calculation of the critical ratios yielded poor coefficients for the 20° points, especially in the left eye ($r = 0.41$ to $r = 0.08$). Otherwise no notable features were revealed.

The spontaneous fluctuation which characterized earlier measures of acuity was evident and a measure of it was extracted from the scores by a method comparable to that previously used (4, 3, 5). The mean percentile steadiness of the total performance of the subjects was about 80. Calculation of the percentile steadiness for the 4 exposure times revealed the percentile steadiness at 1 sec. to be 73; at $\frac{1}{5}$ sec., 77; at $\frac{1}{25}$ sec., 80; and at $\frac{1}{100}$ sec., 87. At the various angular deviations the percentile steadiness at 20° was 67; at 30°, 70; at 40°, 82; and at 50°, 84. The above figures are derived from the scores of both eyes.

Discussion. Since the data here reported were collected by methods used for previous experimentation, differing only by specified experimental conditions, they are comparable to the results of those experiments. The trend in our present data toward poorer acuity with limited exposure time is strengthened by comparison with acuity measures at 30° and 60° on the horizontal meridian with unlimited exposure time (1). Here at 30° the mean acuity in the first eye tested (left) was 1.5 and at 60°, 4.9. In the second eye tested (right) it was 1.1 at 30° and 4.6 at 60°. Both sets of acuities are better than the present comparable measures with 1 sec. exposure. The acuities obtained at the same points with scotopic illumination (2) were 3.7 at 30° and 7.5 at 60° in the first eye (left) and 3.4 at 30° and 7.5 at 60° in the second eye (right). The above 30° measures are most closely comparable to present photopic 30° measures taken at $\frac{1}{25}$ sec. exposure, indicating that, in bright light at 30° on the horizontal meridian, the eye can perceive as much detail in $\frac{1}{25}$ sec. as it can with unlimited exposure in dim light. At 60° the scotopic acuity falls between the present photopic 60° measures taken with 1 sec. and $\frac{1}{5}$ sec. exposure, and may be correspondingly interpreted. Similar comparisons of the present data with other acuity measures may be made by reference to previous publications (3, 4, 5).

The percentile steadiness observed in the present experiment increases as the exposure time is shortened, indicating that whatever factor is responsible for the instability of peripheral perception operates most effectively at longer exposures. However, the above figures do not check well with previous measures which showed a percentile steadiness of 78 with unlimited exposure time, and 80 before training and 79 after training in tests using $\frac{1}{5}$ sec. exposure. The rather sharp rise in percentile steadiness between 30° and 40° in the present group is not understood.

Notable in the present experiment is the lack of any overall tendency to improve through practice from eye to eye. The total scores of the two eyes differ by less than 1%. This contrasts with strong eye-to-eye improvement in experiments in which unlimited exposure time was permitted and supports the pre-

vious conclusion (3) that the subject must have time to work out his impression of the stimulus position if improvement through practice is to be expected.

SUMMARY

(1) Simple form acuity in the retinal periphery was measured in 55 subjects on the horizontal meridian under photopic illumination at 5 angular deviations from the line of central vision with 4 exposure times.

(2) A significant decrease in acuity was caused by both increased angular deviation and decreased exposure time.

(3) Acuity measures obtained in the present experiment are compared with measures previously obtained with comparable technique.

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EFFECT OF PARTIAL AND COMPLETE DESTRUCTION OF THE TACTILE CEREBRAL CORTEX ON CORRECT CONDITIONED DIFFERENTIAL FORELEG RESPONSES FROM CUTANEOUS STIMULATION

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Received for publication September 19, 1947

The purpose of this sixth study on the function of the association cells of the cerebral cortex was to determine the effect of elimination of various portions or all of the cutaneous cerebral cortex on correct, conditioned, differential responses of the foreleg with two different sets of cutaneous analysers. By sensory cortex is meant the portion that has shown voltage changes as a result of tactile stimulation. In the first set of tests the positive- and negative-conditioned stimuli consisted of stroking the left side of the back lightly with a hand brush once per second, with and against the grain, and in the second set of tests, the back was stroked with the grain once and three times per second. The first and usual daily session of tests consisted of 25 trials with the positive-conditioned stimulus and 13 trials with the negative-conditioned stimulus. Occasionally these numbers were reversed. The order of the tests was the same as used in a previous study (9).

Stimuli from other senses were excluded by the same procedures as were used previously. Since hearing should not be impaired by any of these lesions, while cutaneous cerebral impulses might be abolished, all errors for the negative-conditioned stimulus were reprimanded by the command 'No!' Frequently during the retraining period following the operations this command was accompanied by a tap, but the tap was never used alone as it was during the earlier work with smell and hearing. It should be mentioned that every dog used understood the commands 'No' and 'Quiet'. To avoid differences of brush pressure as possibilities for formation of positive- and negative-conditioned reflexes, the pressure of the brush was occasionally varied for a test, but ordinarily pressure was maintained as nearly uniform as possible. Also the same area of the back was stimulated, except when purposely varied for the with- and against-grain stimuli.

With the exception of lesion A (fig. 1, les. A) cortical destruction was accomplished during two unilateral operations. Suction and thermo-coagulation from a high frequency current were the methods ordinarily used. It is obvious from figure 1 that it is difficult to make lesion A without injury to the motor cortex in front, but fortunately for this problem all of the motor cortex situated between the cruciate and postcruciate sulci, with the exception of the foreleg area on the left side, can be destroyed without appreciable effect on the positive-conditioned reflex for the right foreleg. In fact, damage to the motor cortex from the lesions has resulted in some interesting modifications of the original conditioned response of the foreleg. It is likely that all of the lesions extended slightly beyond

the boundaries shown in figure 1, even though the suction tube followed the margins closely and a 2-mm. space was allowed for spread of the current to the border zone.

Lesion A (*fig. 1, les. A*). This area, which occupies all of the space between the postcruciate and ansate sulci, was coagulated in *dogs 1, 2* and *3*, and sucked out in *dogs 4* and *5*. It represents Adrian's tactile center for the leg, trunk and arm for the opposite side of the body, and Woolsey's tactile area I for these structures.

The formalin-preserved brains revealed complete destruction of this area on both sides for each dog. The brain of *dog 3* showed the purposeful destruction of the precentral motor area on the left side. The following additional damage was indicated: *dog 2*, foreleg motor area, left side; *dogs 1* and *3*, hindleg motor

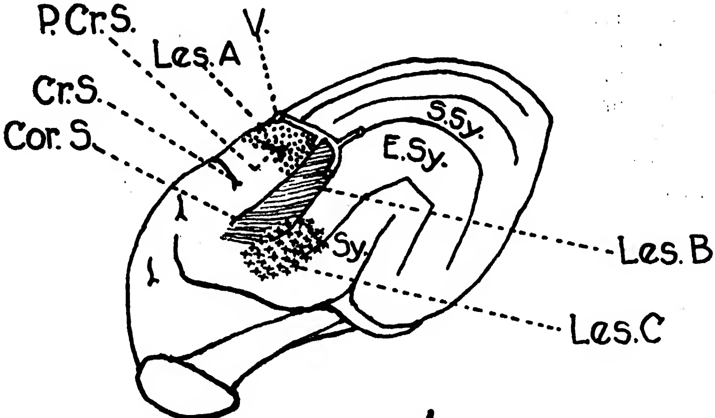

FIG. 1. DOG'S BRAIN showing areas of cortical destruction: lesion A, *dots*; lesion B, *parallel lines*; lesion C, *crosses*. *Abbreviations:* Cor.S., coronal sulcus; Cr.S., cruciate sulcus; E.Sy., ectosylvian gyrus; P.Cr.S., posterior cruciate sulcus; Sy., sylvian gyrus; S.Sy. Suprasylvian gyrus; V. vein.

area, both sides; *dogs 4* and *5*, hindleg motor area, right side; and *dogs 1, 4* and *5*, a narrow strip behind the ansate sulcus. Marchi sections through the medulla showed degeneration in the pyramids. This was considerable on both sides for *dogs 1* and *2*, about complete for *dog 3*, but confined to the right pyramid for *dogs 4* and *5*. Immediately after the operation motor defects were indicated for the right foreleg of *dogs 2* and *3*, both hindlegs for *dogs 1* and *3* and the left hindleg for *dogs 4* and *5*. The cutaneous placing and hopping reflexes were completely eliminated.

RESULTS. Two weeks after the operation *dog 4* responded with the positive-conditioned reflex during the first trial; *dogs 1, 2, 3* and *5* required 9, 2, 7 and 1 trials, respectively, with shock punishment before the reflex returned. It should be mentioned that *dogs 3* and *5* made no responses previously to 5 tests that were

not shocked. The original quick flexion of the foreleg, involving all joints, persisted after the operation with *dogs 1, 4* and *5,* but it was decidedly altered with *dogs 2* and *3.* With *dog 2* the reflex consisted of a stiff leg-shoulder movement. With *dog 3* the movement was produced by a rotation of the body in

TABLE 1. *Conditioned stimuli: brush stroke, with and against grain*

CORTICAL LESIONS	BEFORE OPER., DIFF. FIRST APPEARED, SESSION NO.	AFTER OPER., DIFF. FIRST APPEARED, SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED		TOTAL NUMBER OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSES
			Pos. C to I	*Neg.* I to C	*Pos.* C to I	*Neg.* I to C	
			A				
Dog 1, C	1st	1st	25–0 13–0	0–13 0–25	38–0	0–38	Good
Dog 2[1], C	1st	1st	25–0 13–0	0–13 0–25	74–2	2–75	Good
Dog 3[1], C	1st	1st	25–0 13–0	0–13 0–25	38–0	0–38	Good
Dog 4, S	1st	1st	25–0 13–0	0–13 0–25	38–0	0–38	Good
Dog 5, S	1st	1st	24–1 12–1	0–13 1–24	310–17	18–184	Good
			A and B				
Dog 6, C	1st	1st	25–0 13–0	0–13 0–25	375–15	32–294	Good
Dog 7, S	1st	1st	25–0 13–0	0–13 0–25	194–6	11–133	Good
Dog 8, S	1st	1st	25–0 13–0	0–13 0–25	551–26	16–197	Good
Dog 9, S	1st	1st	24–1 13–0	0–13 2–23	350–21	38–209	Good

Diff. = correct conditioned differential response; Pos. = positive conditioned reflex; Neg. = negative conditioned reflex; Oper. = operations; No. = number; C = coagulated; S = sucked out; C to I = ratio of correct to incorrect responses; I to C = ratio of incorrect to correct responses.

[1] A modified conditioned foreleg response after the lesions.

the harness and no elevation of the foreleg occurred if the neck was not supported from above by a rope attached to the collar. At first these modified responses were apparently elicited with considerable difficulty as indicated by increased tension and considerable delay in starting a much slower movement. *Dog 3*

barked loudly during each test in which there was an absence of foreleg response. Ultimately the altered foreleg reflexes appeared much earlier in the tests and served the purpose of this problem as satisfactorily as the usual foreleg flexion.

It is apparent from column 3 of tables 1 and 2 that *dogs 1* to *5*, inclusive, produced correct, conditioned, differential responses with both sets of cutaneous

TABLE 2. *Conditioned stimuli: slow and fast brush stroke*

CORTICAL LESIONS	BEFORE OPER., DIFF. FIRST APPEARED, SESSION NO.	AFTER OPER., DIFF. FIRST APPEARED, SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED: *Pos.* C to I	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED: *Neg.* I to C	TOTAL NUMBER OF TESTS: *Pos.* C to I	TOTAL NUMBER OF TESTS: *Neg.* I to C	CHARACTER OF CORRECT CON. DIFF. RESPONSES
			A				
Dog 1, C	1st	1st	25–0 13–1	0–13 1–24	38–0	1–37	Good
Dog 2[1], C	1st	1st	25–0 13–0	0–13 0–25	63–0	0–51	Good
Dog 3[1], C	1st	1st	25–0 13–0	0–13 0–25	38–0	0–38	Good
Dog 4, S	1st	1st	25–0 13–0	0–13 0–25	38–0	0–38	Good
Dog 5, S	1st	1st	25–0 13–0	0–13 1–24	152–1	5–90	Good
			A and B				
Dog 6, C	1st	1st	25–0 15–0	0–13 1–24	307–9	35–85	Good
Dog 7, S	1st	1st	25–0 13–0	0–13 0–25	38–0	0–38	Good
Dog 8, S	1st	1st	25–0 13–0	0–13 0–25	73–5	0–76	Good
Dog 9, S	1st	1st	25–0 12–1	0–13 0–25	135–3	11–105	Good

Abbreviations listed and defined under table 1.

[1] A modified conditioned foreleg response after the lesions.

stimuli during the first session of tests that followed the last operation. The first of the two perfect or practically perfect scores shown in columns 4 and 5 of tables 1 and 2 were records for the first daily session for *dogs 2*, *3* and *4* and the 2nd and 5th for *dogs 1* and *5*. The poor showing for *dog 5* can be attributed to the fact that it belonged to the easily inhibited or martyr type. Before the

operation this dog had more difficulty than the others in securing perfect tallies for a daily session of tests. The summations recorded in columns 6 and 7 disclose an absence of lesion A effects.

Lesions A and B (*fig. 1, Les. A and B*). This area, which includes all of the sensory portion of the sigmoid gyrus and the coronal gyrus, was coagulated on both sides in *dog 6* and sucked out on both sides in *dogs 7, 8* and *9*. It represents all of Adrian's tactile center for the cat with the exception of 2 terminals for the feet and all of Woolsey's tactile area I.

The formalin-prepared brains revealed complete destruction to the area on both sides without serious damage to neighboring and deeper parts. The left foreleg area of *dog 8*, however, may have suffered slight injury. Marchi-stained sections through the medulla of these dogs showed some degeneration in the pyramids, being most pronounced in the left pyramid of *dog 8* and the right pyramid of *dog 9*. The immediate motor symptoms were apparently confined to the right foreleg of *dog 8* and the left hindleg of *dog 9*. Cutaneous placing and hopping reflexes were not obtainable for any of these dogs.

RESULTS. Two weeks after the last operation the first positive-conditioned reflex appeared with the 1st trial with *dog 7*, the 2nd with *dog 6*, the 12th with *dog 8* and the 32nd with *dog 9*, and each failure of response was punished by shock. Except for a stiff leg-shoulder movement of the foreleg of *dog 8*, the positive-conditioned reflexes were unaltered. They were at first, however, slower in appearing and slower in movement. With *dog 9* each of the first conditioned stimuli evoked two or more rapid but normal flexions of the foreleg, which were followed or accompanied by a licking of the chops, rapid respiration and other movements suggestive of considerable excitation.

It is obvious from column 3 of tables 1 and 2 that correct, conditioned, differential responses were obtained during the first session of tests after the last operation for all dogs of this group with both sets of cutaneous stimuli. This group of dogs, however, differed from the lesion A dogs in that only a 75 to 80% average of correctness was maintained from the 2nd up to the 10th or 15th daily session of tests, but from this stage on the perfect scores shown in columns 4 and 5 of tables 1 and 2 were readily obtainable. The record of the total number of tests, columns 6 and 7 of tables 1 and 2, suggest a more serious effect of lesions A and B over A alone. In addition, all of the dogs of this group could ultimately make perfect scores to a series of alternated positive- and negative-conditioned tests with either set of cutaneous stimuli when the interval between tests was of 3 seconds' duration.

Lesion C (*fig. 1, les. C*). This small area of about 10 mm. in diameter was bilaterally sucked out in *dogs 10* and *11* and bilaterally extirpated with dogs *12, 13* and *14*. It includes or represents Adrian's second tactile area for the feet, Woolsey's tactile area II and Tunturi's 3rd auditory terminal.

The formalin-prepared brains revealed this region to have been completely destroyed with little or no damage to deeper or adjacent parts. It is possible that in *dog 11*, where the gyri on the left side were not well separated, that the lesion should have extended a little further ventrad. Most of the Marchi-

stained sections through the medulla of these dogs showed some, but not pronounced, degeneration in the pyramids.

None of these dogs exhibited motor symptoms. It was reported previously (9) that *dogs 12* to *14* responded to the cutaneous placing reflex with both forelegs after the lesions. After reviewing my notes for this reflex with these dogs, it can be stated that only the first of Bard's tests was used and the movement came only when the leg reached the level of the table top. After noting this movement or rebound in *dogs 10* and *11* and testing blindfolded with Bard's 2nd and 3rd tests, both before and after the lesions, it was apparent that lesion C abolished the placing and hopping reflexes. However, these dogs could go up and down stairs blindfolded in response to a pull on the leash or the command 'Come on!'

Results. Two weeks after the last operation, all of the dogs of this group were able to respond with the positive-conditioned reflex during the first session of tests. With *dogs 10, 11* and *12* the reflex appeared during the 2nd, 5th and 6th trials, all absences of response being punished by shock. *Dogs 13* and *14*, not previously trained for the positive-conditioned reflex, responded during the 14th and 15th trials, which is within the upper average required for a normal dog.

It is apparent from column 3 of tables 3 and 4 that the bilateral destruction represented by lesion C prevented return of correct conditioned differential responses from the with- and against-grain stimuli with *dogs 10* to *14* inclusive for 22 to 27 daily sessions of tests (836 to 1026 trials) and for 2 to 25 sessions (76 to 940 trials) from the slow- and fast-brush stimuli. It should be mentioned that the slow- and fast-brush tests were not started until completion of the with and against tests and that the one dog which required 940 tests was inhibited with difficulty before the operations. After correct responses had been re-established, it required only 2 to 4 sessions of tests with either set of stimuli to produce the first of the two perfect scores recorded in columns 4 and 5 of tables 3 and 4. This performance is rarely surpassed by the average normal dog. Columns 6 and 7 of tables 3 and 4 not only reveal the difficulties of these dogs in reacquiring these correct, conditioned, differential responses after the lesions, but disclose that nearly all of the negative-conditioned tests resulted in foreleg reflexes. The occasional absence of response for a negative-conditioned test was usually followed or preceded by an absence of response for a positive-conditioned test. After re-establishment of these correct responses, each dog was able to respond correctly to a series of alternated positive- and negative-conditioned tests, when the interval between tests was of 3 seconds' duration.

During the time in which the effects of lesion C prevented correct differential responses from cutaneous stimuli, correct responses were always obtainable from 2 different sets of auditory stimuli. In fact, but one set of tests was required to establish correct responses for each set of stimuli.

Another effect to follow this cortical destruction was that the effective area for stimulation was no longer confined to a small area on one or both sides of the back, but included the entire trunk, shoulder, hip and tail and could also be elicited from a tap of the hand in place of the brush stroke.

TABLE 3. *Conditioned stimuli: brush stroke, with and against grain*

CORTICAL LESIONS	BEFORE OPER., DIFF. FIRST APPEARED, SESSION NO.	AFTER OPER., DIFF. FIRST APPEARED, SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED		TOTAL NUMBER OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSES
			Pos. C to I	*Neg.* I to C	*Pos.* C to I	*Neg.* I to C	
			C				
Dog 10, S	1st	25th	25–0 13–0	0–13 1–24	739–4	445–31	Good
Dog 11, S	1st	24th	25–0 13–0	0–13 0–25	510–33	310–144	Good
Dog 12, E	1st	22nd	25–0 13–0	0–13 0–25	640–23	264–98	Good
Dog 13, E	Not tested	27th	25–0 13–0	1–12 0–25	760–4	412–51	Good
Dog 14, E	Not tested	25th	25–0 13–0	0–13 0–25	765–25	498–92	Good
			A, B and C				
Dog 15[1], S	1st	None 26	24–1 13–0	13–0 24–1	629–36	336–16	No
Dog 16[2], S	1st	None 30	23–2 15–0	11–2 25–0	657–35	428–22	No
Dog 17, S	1st	None 85	25–0 13–0	13–0 25–0	1977–19	1220–3	No
Dog 18, S	1st	None 90	25–0 13–0	13–0 25–0	2215–4	1380–1	No
			B and C				
Dog 19, S	1st	39th	25–0 13–0	0–13 0–25	952–4	574–68	Good
Dog 20, S	1st	28th	25–0 13–0	1–12 0–25	883–22	417–142	Good

Abbreviations listed and defined under table 1.

[1] Dog died from a fall.

[2] Bark used for the positive-conditioned reflex. Epileptic convulsions appeared 3 months after the last operation.

Lesions A, B and C (*fig. 1, les. A, B and C*). This destruction, accomplished on both sides of *dogs 15* to *18*, inclusive, by suction, should eliminate all of the tactile cortex.

The formalin-prepared brains of these dogs revealed complete removal of the cortical area represented by these lesions with suggestive injuries to the precentral motor cortex as follows: *dog 17*, hindleg area, right side; *dog 18*, hindleg area,

TABLE 4. *Conditioned stimuli: slow and fast brush stroke*

CORTICAL LESIONS	BEFORE OPER., DIFF. FIRST APPEARED, SESSION NO.	AFTER OPER., DIFF. FIRST APPEARED, SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED		TOTAL NUMBER OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSES
			Pos. C to I	*Neg.* I to C	*Pos.* C to I	*Neg.* I to C	
			C				
Dog 10, S	1st	8th	25–0 13–0	1–12 0–25	261–2	104–56	Good
Dog 11, S	1st	3rd	30–0 13–0	1–19 0–25	137–29	16–106	Good
Dog 12, E	1st	3rd	25–0 13–0	0–13 0–25	162–1	22–78	Good
Dog 13, E	Not tested	2nd	25–0 13–0	0–13 0–25	88–6	9–56	Good
Dog 14, E	Not tested	25th	25–0 13–0	0–12 0–25	688–0	317–79	Good
			A, B and C				
Dog 16[1], S	1st	None 29	23–2 12–2	12–1 23–2	647–64	328–31	No
Dog 17, S	1st	None 66	25–0 13–0	13–0 25–0	1548–3	972–3	No
Dog 18, S	1st	None 60	25–0 13–0	13–0 25–0	1403–1	875–1	No
			B and C				
Dog 19, S	1st	7th	25–0 13–0	0–13 1–24	268–2	107–85	Good
Dog 20, S	1st	8th	25–0 13–0	0–13 0–25	252–27	65–150	Good

Abbreviations listed and defined under table 1.

[1] Bark used for the positive-conditioned tests. Dog developed epileptic convulsions and the last 13 sessions of tests were taken with the dog under dilantin.

both sides (?) and *dog 16*, foreleg area, left side and hindleg area, both sides. Marchi-stained sections through the medulla showed some degeneration in the pyramids. It was scanty with *dogs 15* and *16*, considerable in the right pyramid

of *dog 17* and almost complete in both pyramids of *dog 16*. Immediately after the last operation, motor symptoms were indicated in the left hindleg of *dog 17*, in both hindlegs of *dog 18* (?) and in the right foreleg and both hindlegs of *dog 16*. The cutaneous placing and hopping reflexes were not obtainable for any of these dogs when blindfolded nor would they go up and down stairs. They were supersensitive to a pinch, electric shock or flea bite on any part of the body. Heat resulted in a good reflex but no cry. There was a tendency for the feet to slip out when turning a corner on a run or during feeding.

RESULTS. Two weeks after the second operation, the positive conditioned reflex returned unaltered during the first session of tests with all dogs of this group but *dog 16*. With *dog 15* the first positive response followed 11 failures of response that were punished by shock and 24 and 6 failures were recorded for *dogs 17* and *18*. It was apparent from the early tests of *dog 16* that injury to the left motor cortex was preventing the right foreleg response. The dog barked loudly with each conditioned stimulus and appeared vexed at the non-movement of the foreleg. At this point it was decided to use the bark for the positive conditioned response instead of transferring the reflex to the opposite foreleg.

It is apparent from column 3 of tables 3 and 4 that correct, conditioned, differential responses were not re-established for *dogs 15*, *16*, *17* and *18* from the with- and against-grain stimulation during 26, 30, 85 and 90 sessions of tests or 1017, 1142, 3219 and 3600 trials, nor afterward from the slow- and fast-brush stimuli for *dogs 16*, *17* and *18*, during 29, 66 and 60 sessions of tests or 1068, 2526 and 2280 trials. All of the scores recorded in columns 4 and 5 of tables 3 and 4 were from the last two sessions and were typical. The summary of all tests, columns 6 and 7 of tables 3 and 4, show that nearly all of the negative-conditioned tests resulted in positive responses. When a negative-conditioned test resulted in an absence of response, it was usually followed or preceded by an absence of response for a positive-conditioned test.

Unfortunately death from a fall 2 months after the last operation eliminated further tests from *dog 15*. *Dog 16*, trained for the bark reflex, was seized with epileptic convulsions 3 months after the second operation and the last 13 sessions of tests with the slow- and fast-brush stimuli were taken with the dog under dilantin. It would seem, however, that 5745 and 5880 tests for *dogs 17* and *18*, taken over an interval of about 6 months, were sufficient to demonstrate permanent abolishment of these reflexes.

After completing 30 or more sessions of tests with *dogs 17* and *18* for each set of cutaneous stimuli and not obtaining any sign of correct responses, they were tested for correct, conditioned, differential responses with two sets of sound stimuli, with the result that the positive-conditioned reflex was acquired with the 3rd and 5th trials, and correct differential responses appeared early in the first session of tests for each dog from each set of auditory stimuli. The few errors that occurred for the negative-conditioned test were reprimanded by the command 'No!' Throughout all of the remaining tests, when correct responses were not obtainable for the cutaneous stimuli, they could always be evoked from either set of auditory stimuli.

Following the second operation it was apparent for all dogs of this group that the site for effective stimulation for the positive-conditioned reflex was no longer confined to one or both sides of the back but could be elicited equally well from any part of the trunk, shoulder, hip or tail and could be produced from the back by a tap of the hand in place of the brush stroke.

Unilateral lesions A, B and C had no effect on the immediate return of correct, conditioned, differential responses with either set of cutaneous stimuli, and it made no difference whether the lesion was on the same or opposite side from stimulation.

Lesions B and C (fig. 1, les. B and C). The cortical areas represented by these lesions were sucked out on both sides with *dogs 19* and *20*, and the formalin-prepared brains disclosed this removal complete without apparent damage to neighboring and deeper parts. Marchi-stained sections through the medulla were not completed in time to be used for showing pyramidal degeneration, but no motor symptoms were observed. The cutaneous placing and hopping reflexes were gone; however both dogs could go up and down stairs when blindfolded by tapping nose on the steps.

Results. Two weeks after the last operation the positive-conditioned reflex appeared unaltered during the first and second trials with *dogs 19* and *20* (single error being reprimanded by command 'Pull up!').

It is apparent from columns 2 and 3 of tables 3 and 4 that before operating *dogs 19* and *20* required but one session of tests to acquire correct, conditioned, differential responses with either set of cutaneous stimuli, while afterward they needed 39 and 28 sessions or 1482 and 1064 trials for the with- and against-grain stimuli and 8 and 7 sessions or 304 and 266 trials with the slow- and fast-brush stimuli. It should be stated that the latter series of tests were not started until the former had been completed. After the first appearance of correct differential responses for the with- and against-grain stimuli, *dogs 19* and *20* required 152 and 228 additional trials, with punishment for errors, to produce the first of the two perfect or practically perfect scores recorded in columns 4 and 5 for a daily session of tests. This is double the number used before the lesions were made. It is obvious that *dog 19* required many more trials than *dog 20* for re-establishment of these correct responses, but this was compensated for by the fact that *dog 20* needed more trials to obtain perfect responses. This greater difficulty on the part of *dog 20* was apparently due to the fact that the wave of inhibition or excitation which followed the negative- or positive-conditioned stimulus lasted for considerable time, apparently making it easy to respond to a series of negative or positive conditioned tests, but difficult to change from one to the other. The summary of all tests given in columns 6 and 7 of tables 3 and 4 shows the difficulties each dog encountered in re-establishment of these correct responses after lesions B and C.

This summary also shows that the errors were largely due to the inability of the dog to withhold the foreleg reflex during a negative-conditioned test or lack of correct inhibition. Following the last operation it was noted for both dogs that the area for effective stimulation for the positive-conditioned reflex was

enlarged to include the entire trunk, shoulder, hip and tail and could be evoked from a tap of the hand on the left side of the back instead of the brush stroke. Both dogs were finally able to respond correctly to a series of alternated positive- and negative-conditioned tests when the interval between tests was of 3 seconds' duration, and correct differential responses were sometimes obtained from stimulations of the right or opposite side of the back than ordinarily used.

After *dog 19* had completed 30 sessions of tests without showing any signs of correct responses from the with and against stimuli, two different sets of auditory, conditioned, differential responses were easily acquired through use of the commands 'Pull up!' and 'No!' for the positive- and negative-conditioned reflexes, and these correct auditory responses were always obtainable throughout the remaining cutaneous tests in which correct responses were unobtainable.

Discussion. Previous work (5, 8) had shown the olfactory positive-conditioned reflex unaffected from bilateral ablation of the amygdaloid nucleus and the pyreform lobe cortex, and the auditory positive-conditioned reflex unaffected from removal of the entire auditory cortex, while these lesions abolished correct, conditioned, differential responses from olfactory and auditory stimuli for hundreds of tests. Also, complete removal of the prefrontal cortex cephalad to the precentral motor area produced no effect on correct, conditioned, differential responses from auditory, cutaneous and optic stimuli (6), but abolished correct responses from olfactory stimuli (4). Since electrical stimulation of the pyriform lobe (7) resulted in voltage changes in an area of the prefrontal lobe from which stimulation has evoked inhibition of movements and respiration, it seems likely that this area is also an activating center for the negative olfactory-conditioned reflex, which may function by preventing the precentral motor cortex from firing or act extrapyramidly on a lower motor nucleus by way of the reticulospinal route as reported (2) for the cerebro-respiratory inhibitory reflex. The following bilateral lesions were reported (4, 8 and 9) to have no effect on correct, conditioned, differential responses: *a*) removal of the hippocampi or large portions of the parietal, temporal (pyriform excepted) and occipital cortex for olfactory stimuli; *b*) elimination of auditory cortical area I or II for auditory stimuli; and *c*) destruction of auditory areas I and II for cutaneous stimuli.

The results of this study are comparable to the preceding in that complete destruction of the cutaneous cortex on both sides by lesions A, B and C, while having little or no effect on the positive-conditioned reflex, abolished correct, conditioned, differential responses with two different sets of cutaneous stimuli for over 5000 tests. The effect of the lesions on the correct responses was identical with the preceding effect of other lesions and stimuli, namely, inability to withhold the foreleg response during a negative-conditioned test or lack of correct inhibition. It is true that an occasional negative-conditioned test resulted in absence of foreleg response, but this was usually followed or preceded by absence of response for a positive-conditioned test. The only noticeable effect of lesions A and B, which destroyed all of Woolsey's tactile area I, was need of more trials for obtaining perfect scores after the first correct responses appeared.

The results from lesion C, which may have included a little more cortex than

Woolsey's tactile area II, are of special interest because correct, conditioned, differential responses ultimately returned with both sets of cutaneous stimuli after hundreds of tests with no sign of correct responses. This suggests that the original circuits for eliciting these correct responses were broken somewhere, but where or in how many places is not known. It is possible if all of the afferent tactile fibers to Woolsey's area I happened to be very superficially located in this region that lesion C could have completely eliminated all tactile impulses to the tactile cortex or the equivalent. It is also possible that this region is a part of an important association or inhibitory area for cutaneous sense as the following quotations from the literature might imply: Ferrier has reported movements of the angle of the mouth from stimulations of this area in the cat and dog, while Mann and Garol (12) obtained arm and leg movements in the cat from like stimulations. A large part of Tower's lateral extrapyramidal motor area and Tunturi's third auditory terminal are included in the area. Olson in a thesis under Tunturi reports that electrical stimulation of several points in this area and many points in the coronal gyrus prevents and completely suppresses foreleg twitches evoked from single shock stimulation of the precentral motor cortex, and Garol's strip area 3 is in close proximity (13). In addition, tactile area II has an advantage over tactile area I in that it can receive impulses from both sides of the body.

The fact that the positive-conditioned reflex was not seriously affected by complete removal of the cerebral cortex of the sense studied has been a puzzle for a long time. It was assumed that other thalamic nuclei completed the circuit to other parts of the cortex until Woolsey, Chang and Bard presented evidence that some sensory impulses can pass directly to the precentral motor cortex.

The observations, that injury to the foreleg motor cortex on one side altered the positive-conditioned reflex on the opposite side so that it was evoked from shoulder or trunk muscles, support a previous study (3) that the presence of one precentral motor cortex is essential for the usual foreleg conditioned reflex.

SUMMARY

Bilaterally, lesions A, B and C produced no noteworthy effect on the cutaneous, positive-conditioned foreleg reflex if the precentral motor cortex was not involved. Injury to the opposite foreleg motor area altered the usual foreleg flexion as follows: *a*) stiff leg-shoulder movement, *b*) leg elevated from rotation of body in harness, and *c*) bark served for response.

Bilateral lesions A or A and B permitted immediate return of correct, conditioned, differential responses with either set of cutaneous stimuli, but after lesions A and B more tests were required to obtain perfect scores.

Following lesion C on both sides, 836 to 1026 tests were required to re-establish the first correct, conditioned, differential reflexes from the with- and against-grain stimuli and a lesser number later from the slow and fast stimuli. Not only were perfect records ultimately obtainable, but perfect scores were recorded for a series of alternated positive- and negative-conditioned tests when the interval between tests was of 3 seconds' duration. The final change from the

first correct response to perfect responses for all tests was usually abrupt, but some dogs had difficulties with this stage.

The results from lesions B and C from 2 dogs were identical to lesion C except that more trials were required to re-establish perfect responses.

Unilaterally, lesions A, B and C permitted immediate return of correct, conditioned, differential responses with both sets of cutaneous stimuli, but bilaterally they abolished these responses with both sets of cutaneous stimuli for over 5000 trials, which extended over a 6-month interval. Meanwhile, correct, conditioned, differential responses were always obtainable from 2 different sets of auditory stimuli.

The chief effect of these lesions was an inability to withhold the foreleg reflex during a negative-conditioned test or lack of correct inhibition. This was accompanied by a great increase in the area from which the positive-conditioned reflex could be elicited.

Suggestions were made in the discussion as to possible places of injury to the circuits required for evoking these correct responses.

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PLASMA Ac-GLOBULIN ACTIVITY[1]

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Received for publication September 15, 1947

It has been shown that plasma contains a factor which accelerates the interaction of prothrombin and thromboplastin (1–5). Many of its properties still remain to be elucidated. In this paper we describe a study of the activity of Ac-globulin in storage plasma. This is of interest because large quantities of stored plasma and blood come to be involved in modern medical practice and it is practical to know whether or not this factor is preserved. The information gained is also of importance in the clarification of fundamental viewpoints concerning factors involved in the blood coagulation mechanism.

Experimental. At the slaughter house bovine blood was mixed with 1.85% potassium oxalate in the respective proportion of 7:1. Only the freely flowing blood from a stab wound was used. Thorough mixing was insured and the oxalated blood was immediately taken to the laboratory and centrifuged. Hematocrit readings were made and analyses for Ac-globulin were performed. The plasma samples were then stored in a refrigerator at 5°C. without any special precautions with regard to sterility. The Ac-globulin activity was measured by the methods already described in detail (6). This method is an adaptation of the two-stage prothrombin analysis of Warner, Brinkhous and Smith (7). It measures the activation rate of purified prothrombin as influenced by the accelerator factor. The method is in a sense a two-stage analysis for Ac-globulin activity.

Results. The results with storage plasma are presented in figure 1. It can readily be seen that this plasma factor is exceptionally stable. Even at the end of 2 weeks it was doubtful whether there was any significant decrease in Ac-globulin activity. Thereafter, a definite decrease could be noted. At the end of 2 months over half of the activity still remained. At that time other decomposition reactions had taken place as was evident by perceptible odors in a number of the samples.

In work of this kind one cannot always be absolutely certain that the methods of analysis employed will reveal the true status. For example, many authors believed that prothrombin is not stable in storage plasma whereas it has now definitely been shown that such conclusions are erroneous (8), the method of analysis employed having been unreliable. In order that we might avoid the possibility of being mislead, it was considered advisable to undertake the partial purification of Ac-globulin from refrigerated plasma and compare the results with those obtained with the use of fresh plasma. Several gallons of oxalated bovine plasma were stored at 5°C. for 18 days. Ac-globulin was then purified

[1] Aided by a grant from the U. S. Public Health Service.

by the methods already described (7). The yield was the same as from fresh oxalated plasma and the purity was also approximately the same. The handling of the material during the purification showed no evidence of any differences between fresh plasma and stored plasma. We have, therefore, not only the evidence from analytical work but also from partial purification procedures.

Another approach to the question was from the standpoint of studying the stability of purified Ac-globulin as compared with purified Ac-globulin mixed with oxalated plasma. A certain neutral solution of purified Ac-globulin pos-

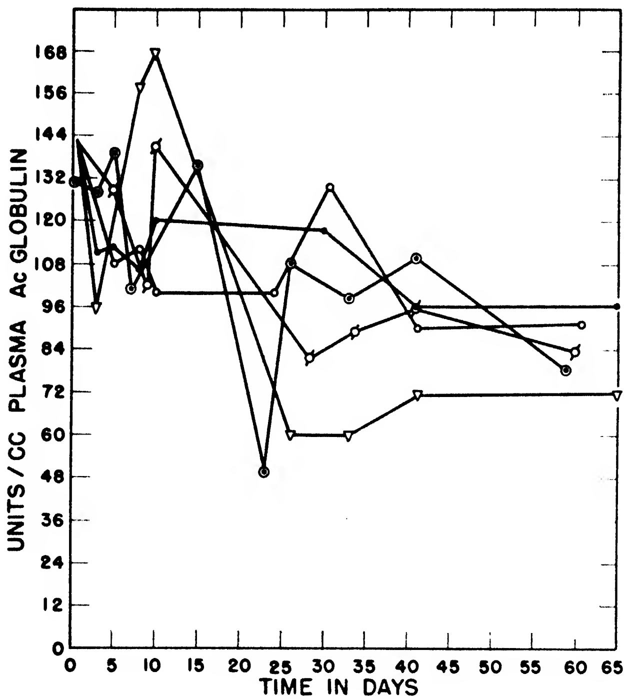

Fig. 1. Ac-globulin activity in oxalated bovine plasma stored at 5°c. The values are corrected for anticoagulant dilution. Each curve represents data for one animal.

sessed a specific activity of 1620 units per cc. It was set at room temperature and at the end of 3 hours only 355 units of activity remained. At the end of 29 hours only 45 units remained. Such instability has been demonstrated many times with other preparations. When such purified material is mixed with oxalated bovine plasma the full titre remains for more than 3 days at 5°C. Measurements beyond that time have not been made. The plasma environment is thus contributory to the stability of this factor.

Some of the first work (1) on the accelerator factor involved the use of $BaCO_3$ adsorption of prothrombin. The accelerator factor was apparently not adsorbed,

whereas prothrombin was completely removed from plasma. Further study of this apparent specificity for prothrombin seemed advisable with the aid of quantitative methods. Oxalated bovine plasma was mixed with various quantities of $BaCO_3$. After 5 minutes a separation was made by centrifugation, and prothrombin analyses were made by the two-stage method. It was found that relatively small amounts of $BaCO_3$ (table 1) completely remove prothrombin whereas the Ac-globulin is unaffected even by extremely large quantities of $BaCO_3$. The figures of table 1 are not corrected for oxalate dilution. This reagent thus furnishes a convenient means for removing prothrombin without changing Ac-globulin activity. This confirms and extends the work of Fantl and Nance (1).

Discussion. Quick (9) was one of the first to show that marked changes can soon be detected in storage plasma by the one-stage method of prothrombin analysis. In the beginning this fact was interpreted to mean that prothrombin disappears rapidly from storage plasma. Later it was reasoned that prothrombin consists of 2 components and only 1 of these, namely, component A, disappears (10). It has, however, been shown that prothrombin does not consist of 2 components (8, 11) and furthermore does not disappear rapidly from refrigerated plasma (12). Quick now presumes that his prothrombin A is identical with the accelerator factor (13). That explanation is also inadequate, because we have shown in the work presented above that the accelerator factor is stable in refrigerated bovine plasma. The changes detected by the one-stage method already appear on the first day and have virtually run their course in 7 days (8). The phenomena described by Quick, therefore, cannot be explained on the basis of Ac-globulin deterioration. Further confirmation of this is indicated by the work of Owren (3), who briefly states that 60 to 70% of the accelerator factor remains at the end of one week. Presumably he was working with human plasma. Our work on human plasma is incomplete, but we are quite certain that there is no loss in activity in 3 days. According to Quick the changes which he observes are already very striking the first day in human plasma (9).

Table 1. *$BaCO_3$ adsorption of Ac-globulin and prothrombin from oxalated bovine plasma*

VOLUME	$BaCO_3$	PROTHROMBIN	AC-GLOBULIN
cc.	*mgm.*	*units/cc.*	*units/cc.*
5	0	286	104
5	75	13	95
5	125	0	92
5	200	0	92
5	250	0	88
5	350	0	95

SUMMARY

Ac-globulin analyses performed on oxalated stored bovine plasma show that Ac-globulin is stable. Partial purification of Ac-globulin can be achieved as

readily with the use of refrigerated plasma as with fresh plasma. The partially purified material is not stable alone but quite stable when dissolved in plasma. $BaCO_3$ adsorbs prothrombin from oxalated plasma, but not Ac-globulin.

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PYRIDOXINE, KETONIC ACIDS, AND SPECIFIC DYNAMIC ACTION

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Received for publication September 2, 1947

In one deamination category, called *oxidative deamination*, the amino group is transformed into urea which is then eliminated.

In another deamination category, called *transamination* (1), the amino group is not eliminated but is transferred from the amino acid to an α-ketonic acid such as pyruvic or oxaloacetic acid, which in turn becomes an amino acid, thus remaining in the service of the body.

The oxidative deamination type of reaction is irreversible, and it is reasonable to assume that its specific dynamic action (SDA) is considerable, reflecting the heat production of the deamination and of the associated processes; the transamination type of reaction is reversible, and it is reasonable to assume that its SDA is slight. The thermodynamically confusing variability in SDA of amino acids may thus reflect differences in the nature of deamination: whether it is oxidative deamination with a high heat increment or whether it is transaminative deamination with a low heat increment.

It is significant that the amino acids which had been reported by Lusk and associates (2) to have no SDA are also the ones that are now believed to undergo transamination most readily and to have the highest transamination coefficients. These are dicarboxylic acids, glutamic and aspartic, and others having similar electrostatic configurations, such as tyrosine.

The extent of transamination depends not only on the electrostatic configuration of the amino acid but also on the presence of proper catalytic systems, namely, apo-transaminases and co-transaminases, and also amino acceptors, namely, α-ketonic acids such as pyruvic acid; or glucose, which is oxidized to pyruvic acid. The co-transaminase is a derivative of the vitamin pyridoxine (1). It is, therefore, reasonable to assume that the addition of pyridoxine and the addition of amino acceptors (pyruvic acid) would lower the SDA of the amino acids which tend to undergo transamination.

METHODS AND RESULTS. To test the above assumptions glutamic acid and tyrosine were fed with and without pyridoxine and with and without pyruvic acid with the effects on SDA (oxygen consumption) shown in figure 1.

The sodium salts of the amino acids were administered to 150–200 gram female rats with a blunt needle and record syringe. Glucose solution (about 3.5 cc) was similarly administered. The oxygen consumption was measured at 26°C. in an 8-chamber Regnault-Reiset-Kleiber type of apparatus (3). Each data point in figure 1 represents the average on 8 rats.

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The nutrients indicated in figure 1 were fed following fast of 12 hours. It is assumed that fasted animals suffer from a *relative* pyridoxine deficiency in the presence of temporary excess amino acid. This observation on the depression of SDA of glutamic acid by pyridoxine on *fasting* animals has been substantiated by observations on the SDA of glutamic acid fed to rats maintained on a *pyridoxine-deficient* diet (4).

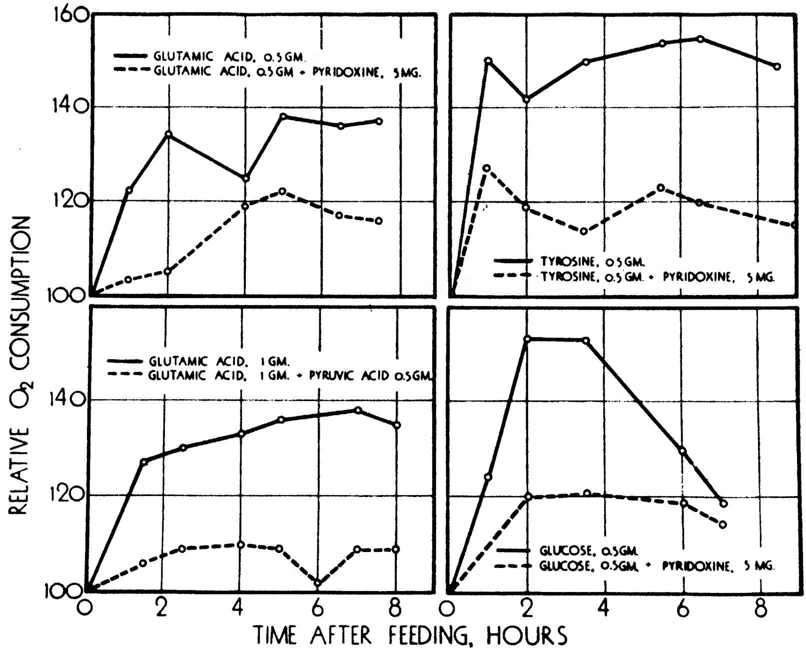

Fig. 1. Upper left: the SDA of glutamic acid is lowered by the addition of pyridoxine. Upper right: the SDA of tyrosine is lowered by the addition of pyridoxine. Lower left: the SDA of glutamic acid is lowered by the addition of pyruvic acid. Lower right: the SDA of glucose is lowered by the addition of pyridoxine, presumably because glucose yields pyruvic acid, which acts as an amino accepter in transamination. Additional charts will become available showing the effect of pyridoxine on the SDA of glycine and of the effect of glutamic acid added to pyridoxine-deficient diets (4) when transamination is presumably at a minimum.

SUMMARY

1. The confusing variability in the specific dynamic action (SDA) of some amino acids appears to be due in part to differences in the nature of deamination, whether it is oxidative, associated with a large heat increment or transaminative, with a small heat increment.

2. The addition of pyridoxine to the diet of fasted rats, or to the diet of rats maintained on a pyridoxine-deficient diet, and the addition of pyruvic acid,

lowers the SDA of the transaminatically active glutamic acid and tyrosine but apparently not of the transaminatically inactive glycine (4).

3. The SDA of glucose is apparently lowered by the addition of pyridoxine, presumably because glucose yields pyruvic acid, which acts as an amino acceptor in transamination.

The SDA of an amino acid thus appears to depend not only on its nature, but also on the presence of pyridoxine, which functions as a prosthetic group in the catalytic system for transamination, and on the presence of α-ketonic acids, such as pyruvic acid, which function as amino acceptors. In general, pyridoxine appears to lower the SDA of foods, especially in rapidly growing and metabolizing tissues.

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EFFECT OF CONVULSANT AND ANTICONVULSANT AGENTS ON ACETYLCHOLINE METABOLISM (ACTIVITY OF CHOLINE ACETYLASE, CHOLINESTERASE) AND ON SENSITIVITY TO ACETYLCHOLINE OF EFFECTOR ORGANS[1]

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Received for publication August 28, 1947

Acetylcholine applied locally to the motor areas of the cerebral cortex or administered parenterally (1–7) induces changes in the electroencephalogram resembling those observed during 'grand mal' attack and the convulsions induced by electric shocks and other convulsion-inducing agents.

In the following it was ascertained whether or not the convulsion-inducing agents *a*) increase the synthesis of acetylcholine (increased activity of choline acetylase), *b*) decrease the hydrolysis of acetylcholine (decreased activity of cholinesterase), and *c*) increase the sensitivity of effector organs to acetylcholine, since these measures, together or separately, may induce a temporary accumulation of acetylcholine. It was also ascertained whether or not the anticonvulsant agents *a*) decrease acetylcholine synthesis, *b*) increase the hydrolysis of acetylcholine, and *c*) decrease the sensitivity of effector cells to acetylcholine, and, if these effects exist, whether or not they parallel the effectiveness of the convulsion-inducing and anticonvulsant agents.

EXPERIMENTAL. *Effects of the substances on acetylcholine synthesis.* The effects of the substances on acetylcholine synthesis were investigated by using *a*) a non-concentrated choline acetylase system and *b*) a concentrated choline acetylase system.

In experiments with non-concentrated choline acetylase, a modified method of Quastel, Tennenbaum and Wheatley (8, 9) was used. Mixtures containing varying amounts of the substances (pH adjusted to 7.4), 100 mgm. frog brain homogenized by means of the apparatus of Potter and Elvehjem (10), 3 mgm. physostigmine salicylate and 3 cc. Ringer's solution were shaken and were incubated aerobically for 4 hours at 37°C. After incubation the amount of acetylcholine synthesized was assayed biologically on the sensitized rectus abdominis muscle of the frog.

Incubated mixtures containing only frog brain, physostigmine salicylate and Ringer's solution served as controls. It was ascertained whether or not the substances modified the sensitivity of the rectus abdominis muscle to the acetylcholine content of the mixtures during the two minutes of immersion for the biological assay by adding the substances to incubated control mixtures after incubation. If the substances modified the sensitivity of the rectus abdominis muscle to acetylcholine, the changes were accounted for in the calculation.

[1] This study was aided by a grant from the John and Mary R. Markle Foundation.

The amount of acetylcholine synthesized was calculated by subtracting from the acetylcholine content of incubated mixtures the acetylcholine content of identical non-incubated mixtures. The amount of acetylcholine synthesized in the control mixtures containing only brain, physostigmine salicylate and Ringer's solution was taken as 100%. The acetylcholine content of the mixtures containing the various substances used was expressed as a percentage of the control. All results deviating from 100% by more than twice the square root of the sum of the squares of the standard error of the mean of the controls and the standard error of the mean of the experiments were considered significant.

TABLE 1. *Effect of the substances on acetylcholine synthesis*

SUBSTANCE	AMOUNT OF ACETYLCHOLINE SYNTHESIZED,[1] % OF CONTROL				
	Mol. concentration of the substances added to 100 mgm. homogenized brain				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Hydantoin	73 ±2.1	76 ±1.9	80 ±2.2	86 ±1.1	
Methyl-phenyl-ethyl hydantoin	50 ±2.5	70 ±2.0	89 ±0.9	98 ±0.6	
Diphenylhydantoinate sodium	35 ±3.6	47 ±3.3	70 ±1.3	84 ±0.6	
Trimethadione	102 ±0.8	105 ±1.0	108 ±1.1	96 ±0.8	
Sodium bromide	112 ±0.7	116 ±1.3	101 ±0.7	106 ±1.0	
Phenobarbital	57 ±3.0	70 ±2.5	85 ±1.1	107 ±1.4	
Barbital sodium	52 ±3.5	90 ±1.1	102 ±0.8	111 ±0.4	
Pentobarbital sodium	48 ±3.3	84 ±2.0	99 ±1.0	106 ±1.1	112 ±1.7
Diallylmalonylurea	37 ±2.0	52 ±3.0	57 ±1.3	113 ±1.1	
Iso-amyl-ethyl-barbiturate sodium	26 ±1.9	43 ±2.2	88 ±0.5	106 ±0.9	
Pentamethylene tetrazol	107 ±2.0	168 ±3.9	151 ±3.0	114 ±1.2	
Picrotoxin		61 ±2.1	81 ±1.8	122 ±2.3	132 ±1.8
Digitoxin			38 ±3.5	57 ±2.5	105 ±1.3
Scilliroside			50 ±2.6	50 ±1.9	68 ±3.1
Strychnine		6 ±2.0	46 ±1.4	91 ±1.7	102 ±1.1
Morphine		66 ±1.9	90 ±1.1	99 ±1.0	99 ±0.6
Camphor	23 ±3.4	70 ±1.9	91 ±1.6	104 ±2.0	

[1] In this and following tables each value represents the mean of 10 separate experiments and the standard error of the mean.

It was found that the amount of acetylcholine synthesized increased in the presence of pentamethylene tetrazol and picrotoxin, was not significantly modified in the presence of sodium bromide and trimethadione, and was decreased in the presence of the other substances used (for strychnine and morphine see also 11, for camphor see also 12, (table 1).

In experiments with concentrated choline acetylase the effect of the substances on acetylcholine synthesis was investigated using a concentrated enzyme preparation obtained from acetone-dried brain as described by Nachmansohn and John (13). The results were similar to the results found in the previous experiments with non-concentrated enzyme, indicating that the substances tested exerted their effects by changing the activity of choline acetylase.

Effect of the substances on cholinesterase. An accumulation of acetylcholine in the brain (as suspected in the presence of the convulsant agents) may occur, not only because of an increased acetylcholine synthesis, but also through a decreased hydrolysis of acetylcholine. An unusual decrease of the acetylcholine content (as suspected in the presence of the anticonvulsant agents) may occur, not only because of a decreased synthesis but also through an increased hydrolysis of acetylcholine. The effect of the substances on the activity of cholinesterase was investigated following *a*) a modified method of Glick (14, 15) and *b*) a manometric method (16). Frog brain, homogenized with the apparatus of Potter and Elvehjem (10), and human serum served as sources of cholinesterase.

With the modified method of Glick, 1 cc. of an enzyme suspension, 1 cc. of a buffer solution containing 0.8 gram acetylcholine bromide per 100 cc. (or 0.657 gram of acetylcholine chloride in the experiments testing the effect of sodium bromide) and 1 cc. of a buffer solution containing the substances to be tested in varying concentrations (pH corrected to 8, wherever necessary) were shaken and incubated at 37°C. for half an hour. A bicarbonate buffer solution of pH 8 was used instead of the customary veronal buffer solution, since the effect of the barbiturates on the activity of cholinesterase was one of the subjects under investigation. The cholinesterase activity was terminated at the end of the half-hour incubation period by addition of 5 cc. of an aqueous solution containing 0.1 gram physostigmine salicylate per 100 cc. Half a cc. of an indicator solution containing 0.04 gram bromthymol blue per 100 cc. was added to each sample and the color developed compared by means of a photoelectric colorimeter with standard solutions consisting of 3 cc. of the buffer solution containing acetic acid in various concentrations, 5 cc. of the aqueous physostigmine salicylate solution and 0.5 cc. of the bromthymol blue solution.

Mixtures containing the enzyme preparation, acetylcholine and buffer solution, incubated for half an hour at 37°C., served as controls. To ascertain whether the convulsant and anticonvulsant agents develop acid or alkali in the absence of acetylcholine during the half-hour incubation, another series of mixtures was also incubated containing enzyme, buffer and the convulsant and anticonvulsant agents in varying concentrations. After half an hour of incubation, 1 cc. of an acetic acid solution containing a known amount of acetic acid dissolved in the buffer solution, 5 cc. of the physostigmine salicylate solution and 0.5 cc. of the bromthymol blue solution were added and the acid content was determined. Any changes in the amount of acid due to the presence of the convulsant and anticonvulsant agents were taken into account during the calculation. The amount of acid developed in the presence of the convulsant and anticonvulsant agents was expressed as a percentage of the amount of acid developed in the controls containing enzyme, acetylcholine and buffer solution.

With the manometric method, either *a*) 0.5 cc. of homogenized frog brain suspended in bicarbonate Ringer's solution at pH 7.4, or *b*) 0.5 cc. of human serum, 1 cc. of a bicarbonate Ringer's solution containing the convulsant and anticonvulsant agents in varying concentrations (pH corrected to 7.4, wherever necessary) and 1.5 cc. of bicarbonate Ringer's solution were placed in the center

part of the vessel of the Warburg apparatus and 0.3 cc. of a bicarbonate Ringer's solution containing 5 grams acetylcholine bromide in 100 cc. (or acetylcholine chloride wherever the effect of sodium bromide was tested) was placed in the side arm. The activity of cholinesterase was then ascertained manometrically by measuring the amount of CO_2 liberated during the hydrolysis of acetylcholine.

Incubated mixtures containing the enzyme preparation, acetylcholine and bicarbonate Ringer's solution alone served as controls. Calculations were made as above.

TABLE 2. *Effect of the substances on the activity of cholinesterase (modified method of Glick)*

	ACTIVITY OF CHOLINESTERASE IN % OF CONTROL							
	Enzyme from human serum				Enzyme from frog brain			
	Mol. concentration of the substances used							
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Hydantoin	100 ±1.1	100 ±0.4	102 ±0.7	99 ±0.5	101 ±0.4	100 ±1.0	99 ±1.0	98 ±0.3
Methyl-phenyl-ethyl hydantoin	87 ±1.2	97 ±1.3	100 ±0.5	100 ±1.0	83 ±1.6	96 ±0.4	98 ±1.1	100 ±1.3
Diphenylhydantoinate sodium		100 ±1.0	101 ±1.1	99 ±0.5	94 ±0.6	95 ±0.9	96 ±1.2	99 ±0.4
Trimethadione	99 ±1.1	97 ±0.4	101 ±0.8	100 ±1.4	100 ±1.7	104 ±0.6	100 ±0.5	96 ±1.6
Sodium bromide	137 ±2.2	105 ±1.3	98 ±0.9	99 ±0.5	134 ±2.5	108 ±0.5	99 ±1.1	100 ±1.4
Phenobarbital	96 ±1.8	99 ±0.4	99 ±1.4	98 ±1.1	95 ±2.5	97 ±0.7	102 ±0.9	100 ±0.7
Barbital sodium		96 ±2.0	97 ±2.4	100 ±0.3		94 ±2.6	95 ±1.9	97 ±1.9
Pentobarbital sodium		95 ±1.8	97 ±2.0	100 ±1.0		95 ±2.3	99 ±2.0	99 ±1.1
Diallylmalonylurea	100 ±1.7	100 ±0.6	90 ±0.4	100 ±0.2	93 ±3.2	100 ±2.0	102 ±1.5	103 ±0.9
Iso-amyl-ethyl-barbiturate sodium		96 ±0.3	100 ±1.0	100 ±1.3		94 ±1.7	98 ±1.1	99 ±0.4
Pentamethylene tetrazol	86 ±2.3	92 ±1.0	97 ±0.9	97 ±0.4	86 ±1.0	88 ±1.4	97 ±1.5	100 ±0.4
Picrotoxin		76 ±1.7	103 ±1.4	101 ±1.1		78 ±1.3	107 ±0.8	101 ±1.0
Digitoxin		105 ±0.9	102 ±0.4	102 ±1.3		107 ±1.4	102 ±1.1	102 ±0.7
Scilliroside		74 ±2.0	85 ±0.6	95 ±0.7		71 ±2.2	93 ±1.1	95 ±1.3
Strychnine		58 ±3.2	77 ±2.1	90 ±1.0		65 ±3.1	73 ±2.3	83 ±1.5
Morphine		72 ±2.9	87 ±1.7	98 ±1.0		75 ±2.7	84 ±1.6	93 ±0.6
Camphor	100 ±1.0	101 ±0.4	100 ±0.3	102 ±0.6	103 ±1.5	96 ±1.0	97 ±0.6	102 ±0.4
Physostigmine				5 ±1.0				25 ±1.9

The activity of cholinesterase decreased in the presence of strychnine, morphine, scilliroside (17), pentamethylene tetrazol, picrotoxin and methyl-phenyl-ethyl hydantoin (the most potent inhibitor being strychnine), was slightly increased in the presence of high concentrations of sodium bromide and was not modified in the presence of the other substances used (table 2). The results found using the manometric method were similar to those reported in table 2. Similar results have already been reported for strychnine (18–20), morphine (21–26), some of the barbiturates (27) and digitoxin (28).

Effect of the substances on acetylcholine sensitivity of striated muscle. Striated muscle is a sensitive indicator of the responsiveness to acetylcholine of effector organs within the cholinergic system. Therefore, changes in acetylcholine sensitivity of striated muscle induced by the various agents used may serve as an indicator of changes in acetylcholine sensitivity of other tissues.

In the following, quantitative relationships were established between the effect of agents modifying the activity of cholinesterase and the acetylcholine sensitivity of striated muscle. It was also ascertained whether or not agents not modifying the activity of cholinesterase modify the sensitivity of striated muscle to acetylcholine.

The effects of the substances on the response of striated muscle to acetylcholine were investigated according to a method described previously (29). Shortening of the rectus abdominis muscle was induced by immersion in a Ringer's solution containing acetylcholine bromide, 50 μg per 100 cc., or acetylcholine chloride in the experiments testing the effect of sodium bromide, for 2 minutes. In 2 minutes the muscle reached almost maximum shortening. The shortening of the muscle was registered by an isotonic lever on a kymograph. After stabilization, between two shortenings induced by the acetylcholine solution, instead of washing with Ringer's solution for 10 minutes, the muscle was washed for 5 minutes and immersed for 5 minutes in Ringer's solution containing one of the

TABLE 3. *Effect of the substances on the response of muscle to acetylcholine*

SUBSTANCE	MUSCLE SHORTENING IN % OF CONTROL					
	Concentration of the substances in mols					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Hydantoin	77 ±2.1	81 ±1.6	104 ±0.1	104 ±1.0	103 ±0.8	97 ±0.5
Methyl-phenyl-ethyl hydantoin	24 ±4.1	82 ±1.1	102 ±1.2	102 ±0.5	105 ±1.0	108 ±0.9
Diphenylhydantoinate sodium	72 ±2.3	76 ±2.0	85 ±0.7	100 ±0.3	103 ±0.1	101 ±0.4
Trimethadione	111 ±0.6	101 ±0.2	108 ±1.5	110 ±2.0	110 ±2.2	105 ±0.7
Sodium bromide	74 ±1.1	96 ±0.5	102 ±0.6	104 ±0.7	101 ±0.3	103 ±1.0
Phenobarbital	104 ±1.4	114 ±0.9	121 ±1.4	117 ±0.8	112 ±1.3	105 ±0.2
Barbital sodium	100 ±0.4	97 ±0.8	99 ±0.2	100 ±1.0	100 ±0.4	99 ±0.7
Pentobarbital sodium	s[1]	29 ±4.0	82 ±1.3	102 ±0.8	101 ±0.6	103 ±1.1
Diallylmalonylurea	110 ±1.1	127 ±1.3	129 ±0.5	122 ±0.4	118 ±1.1	114 ±0.8
Iso-amyl-ethyl-barbiturate sodium	s[1]	32 ±4.3	98 ±1.7	122 ±0.5	116 ±0.7	106 ±0.6
Pentamethylene tetrazol	340 ±5.0	130 ±2.7	106 ±1.3	100 ±1.1	102 ±0.4	101 ±0.9
Picrotoxin		234 ±5.3	142 ±3.4	108 ±2.5	104 ±0.7	106 ±1.4
Digitoxin			103 ±0.3	105 ±0.6	104 ±1.2	103 ±2.0
Scilliroside		14 ±3.7	37 ±3.5	101 ±1.6	121 ±1.9	104 ±1.1
Strychnine		s[1]	10 ±3.5	15 ±3.2	47 ±2.2	84 ±2.3
Morphine		129 ±0.7	98 ±1.1	99 ±1.4	101 ±0.5	100 ±1.3
Camphor	20 ±3.9	24 ±3.6	43 ±2.8	85 ±2.2	100 ±1.1	102 ±0.4
Physostigmine			492 ±12.7	276 ±7.7	164 ±3.8	110 ±0.3

[1] s means shortening of the muscle which occurred during the immersion of the muscle in the solution for 5 minutes, without addition of acetylcholine.

substances to be tested (pH 7). For controls, muscles were immersed only in Ringer's solution and shortening of the muscle was induced with the acetylcholine solution as described above. The repeatedly induced shortenings, each lasting 2 minutes, were of the same magnitude for at least 3 hours. This period of time was longer than the duration of the experiments described.

The amount of shortening of muscle after immersion in the solution of the compounds was expressed as a percentage of the amount of shortening (induced

TABLE 4. *Effect of the substances on the response of the rectus abdominis muscle to potassium*

SUBSTANCE	MUSCLE SHORTENING, IN % OF CONTROL: Concentration of the substances in mols					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Hydantoin	84 ±2.1	109 ±2.7	131 ±2.2	120 ±1.1	111 ±2.3	107 ±2.5
Methyl-phenyl-ethyl hydantoin	101 ±1.8	120 ±1.1	126 ±1.5	129 ±0.7	126 ±1.3	116 ±0.9
Diphenylhydantoinate sodium	17 ±3.4	45 ±3.6	62 ±3.9	68 ±2.8	72 ±2.0	100 ±1.7
Trimethadione	94 ±0.7	106 ±1.5	107 ±1.9	106 ±1.3	105 ±1.4	105 ±2.2
Sodium bromide	100 ±1.7	106 ±0.9	113 ±0.5	116 ±1.1	106 ±1.5	105 ±0.8
Phenobarbital	80 ±2.0	131 ±2.1	158 ±3.2	152 ±3.0	135 ±2.0	120 ±2.2
Barbitol sodium	207 ±4.8	123 ±1.7	119 ±0.7	115 ±1.3	108 ±1.6	106 ±0.7
Pentobarbital sodium	s[1]	45 ±1.8	94 ±1.4	124 ±1.5	120 ±1.7	102 ±0.3
Diallylmalonylurea	50 ±3.5	116 ±1.6	136 ±1.3	126 ±1.7	118 ±1.2	111 ±0.6
Iso-amyl-ethyl-barbiturate sodium	s[1]	49 ±3.5	90 ±1.3	104 ±1.2	102 ±1.1	100 ±2.0
Pentamethylene tetrazol	160 ±2.8	132 ±1.7	110 ±2.0	104 ±0.5	103 ±0.8	104 ±1.1
Picrotoxin			122 ±1.4	125 ±1.3	112 ±1.5	103 ±1.0
Digitoxin			135 ±2.0	134 ±1.1	131 ±1.5	112 ±1.4
Scilliroside		270 ±5.4	310 ±6.7	233 ±5.8	173 ±3.6	134 ±1.9
Strychnine		s[1]	69 ±2.3	96 ±1.0	107 ±1.1	112 ±0.6
Morphine		139 ±1.1	144 ±1.5	141 ±1.7	138 ±1.4	133 ±2.1
Camphor	10 ±4.2	37 ±2.2	82 ±1.7	123 ±2.0	126 ±0.7	125 ±2.0
Physostigmine				133 ±0.9	131 ±1.9	123 ±1.1

[1] s means shortening of the muscle, which occurred during the immersion of the muscle in the solution for 5 minutes, without addition of potassium.

by acetylcholine) of the same muscle before immersion in the solution of the compounds.

The response of the rectus abdominis muscle to acetylcholine was increased by pentamethylene tetrazol, picrotoxin, morphine, physostigmine and to a small extent by phenobarbital, diallylmalonylurea and iso-amyl-ethyl-barbiturate sodium. The acetylcholine sensitivity of the muscle decreased in the presence of sodium bromide, hydantoin, methyl-phenyl-ethyl hydantoin, diphenylhydantoinate sodium, iso-amyl-ethyl barbiturate (higher concentrations), pentobarbi-

tal sodium, scilliroside, strychnine and camphor. The acetylcholine sensitivity was not modified in the presence of trimethadione, barbital sodium and digitoxin (table 3). An increase of the acetylcholine sensitivity in the presence of morphine (30–32), physostigmine (33) and pentamethylene tetrazol (34, 35) and a decrease of the acetylcholine sensitivity in the presence of strychnine (32, 36, 37) have already been observed.

Effect of the substances on potassium sensitivity of striated muscle. To ascertain whether the substances modified the sensitivity of muscle to chemical stimuli other than acetylcholine, the effect of the substances on the shortening of muscle induced by a 20 mM KCl solution instead of an acetylcholine solution was also determined.

The substances increased the potassium sensitivity of the muscle, if used in lower concentrations, and decreased the potassium sensitivity if used in higher concentrations. Strychnine and diphenylhydantoinate sodium did not increase the potassium sensitivity, if used in lower concentrations, and decreased it if used in higher concentrations. The potassium sensitivity increased without being followed by a decrease in the presence of sodium bromide, picrotoxin, digitoxin, morphine, hydantoin, methyl-phenyl-ethyl hydantoin, pentamethylene tetrazol and physostigmine and was not modified in the presence of trimethadione (table 4).

Muscle shortening occurred in the presence of high concentrations of strychnine, pentobarbital sodium, and iso-amyl-ethyl-barbiturate sodium (table 3, 4) even in the absence of potassium or acetylcholine.

Discussion. The results indicate that most of the convulsion-inducing agents cause an accumulation or sudden increase of acetylcholine either by increasing the acetylcholine synthesis (pentamethylene tetrazol, picrotoxin) or by decreasing the hydrolysis of acetylcholine (strychnine, morphine). That an accumulation of acetylcholine may be significant in inducing convulsions is further suggested by the observations that the effect of convulsion-inducing agents can be potentiated by simultaneous administration of acetylcholine or physostigmine (38, 39). However, in the case of strychnine (40) for instance, it is difficult to demonstrate (perhaps for technical reasons) an accumulation of acetylcholine in the brain.

The results obtained with striated muscle suggest that the acetylcholine sensitivity of effector organs may increase in the presence of some of the convulsion-inducing agents. The increased acetylcholine sensitivity is due to *a*) a decreased activity of cholinesterase (table 2) and *b*) involvement of other processes, since the agents also increase the potassium sensitivity of the muscle (table 4) and it is known that pentamethylene tetrazol significantly increases the acetylcholine sensitivity of fully eserinized muscle (31).

Convulsions have been observed after administration of camphor and the digitalis glucosides (41), although these agents depress acetylcholine synthesis. Furthermore, they do not increase the sensitivity of effector organs to acetylcholine (table 3), and, except scilliroside in relatively high concentrations, the digitalis glucosides (28) and camphor (table 2) do not decrease the hydrolysis

of acetylcholine. These data would lead to the inference that an accumulation of acetylcholine is not essential for the occurrence of convulsions.

The anticonvulsant agents, with the exception of sodium bromide and trimethadione, decreased the synthesis of acetylcholine, but this decrease was not greater than the decrease of acetylcholine synthesis in the presence of digitalis glucosides and strychnine. The hydrolysis of acetylcholine did not increase in the presence of the anticonvulsant agents, except for large concentrations of sodium bromide (table 2), an effect that would lead to a reduction in the amount of the acetylcholine content of the brain. Also, the acetylcholine sensitivity of the effector organs did not decrease in the presence of the anticonvulsant agents except when used in large concentrations (table 3).

In short, an accumulation of acetylcholine in the central nervous system readily induces convulsions *a*) as shown by local application or parenteral administration of acetylcholine; *b*) acetylcholine increases the effectiveness of other agents in inducing convulsions; *c*) convulsions are induced by agents that increase the sensitivity of effector cells to acetylcholine; *d*) convulsion-inducing agents usually favor an accumulation of acetylcholine either by increasing acetylcholine synthesis or by decreasing acetylcholine hydrolysis.

An accumulation of acetylcholine does not seem to be the only essential factor in the precipitation of convulsions, since *a*) there are convulsion-inducing agents that decrease acetylcholine synthesis without decreasing acetylcholine hydrolysis (digitalis glucosides, camphor); *b*) a decrease of the acetylcholine content of brain was found after administration of strychnine (40); *c*) the ability of agents to increase acetylcholine synthesis (42) often does not parallel their effect in inducing convulsions (43, 44); and *d*) the ability of agents to decrease acetylcholine synthesis or acetylcholine accumulation does not parallel their effectiveness as anticonvulsant agents.

Although acetylcholine may precipitate convulsions or may augment the effect of convulsion-inducing agents and processes, it does not seem evident, from the data available, that disturbances in acetylcholine metabolism leading to acetyl choline accumulation in the central nervous system is per se the primary factor to the induction of fits.

SUMMARY

1. The effect of convulsion-inducing and anticonvulsant agents on acetylcholine synthesis, acetylcholine hydrolysis and on the acetylcholine and potassium sensitivity of striated muscle was investigated.

2. Pentamethylene tetrazol and picrotoxin increased the acetylcholine synthesis, sodium bromide and trimethadione did not modify it and scilliroside, digitoxin, camphor, morphine, strychnine, the barbiturates and hydantoin and its derivatives decreased the acetylcholine synthesis.

3. Strychnine, morphine, scilliroside, pentamethylene tetrazol, picrotoxin, and methyl-phenyl-ethyl hydantoin decreased the activity of cholinesterase; sodium bromide, in concentration of 10^{-2} Mol., increased it; and the other substances did not modify the activity of cholinesterase (obtained from brain and serum).

4. The acetylcholine sensitivity of striated muscle, used as an indicator of the acetylcholine sensitivity of effector organs in the cholinergic system, increased in the presence of pentamethylene tetrazol, picrotoxin, morphine and to a small extent in the presence of phenobarbital, diallylmalonylurea and iso-amyl-ethyl-barbiturate sodium; the acetylcholine sensitivity was not modified in the presence of trimethadione, barbital sodium and digitoxin, and decreased in the presence of the other substances.

5. The potassium sensitivity of striated muscle usually increased in the presence of lower concentrations of the substances used and often decreased in the presence of higher concentrations of the substances.

6. The results suggest that although an accumulation of acetylcholine may precipitate convulsions, it is per se not the primary factor in the induction of fits.

The authors wish to express their gratitude to Dr. C. R. Noller for the generous supply of the scilliroside, to Abbott Laboratories for the trimethadione and to Sandoz Chemical Works, Inc. for the methyl-phenyl-ethyl hydantoine.

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PROTECTION OF THE CEREBRAL CIRCULATION BY THE CEREBROSPINAL FLUID UNDER THE INFLUENCE OF RADIAL ACCELERATION[1]

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Received for publication January 28, 1947

During a change in velocity or direction of motion, forces may act upon the body which are many times the force of gravity When an accelerative force increases the effective weight of a body threefold, it is three times as powerful as the force of gravity (3*g*). Such forces, produced during radial acceleration, become a serious problem during violent maneuvers in high-speed aircraft. Centrifugal forces produced during standard maneuvers act from head to seat (positive *g*). Conversely, forces may act from seat to head (negative *g*) during inverted flying or outside loops.

Wood *et al.* (1) reviewed recent investigations of the physiological effects of positive radial acceleration. However, negative radial acceleration has been considered highly dangerous to both man and aircraft, and, for that reason, relatively little attention has been directed to the effects of negative *g*. In 1877 Salathe (2) concluded from experiments conducted on a small centrifuge that rabbits survived at least twice as long when exposed to negative *g* as during positive *g* of the same magnitude. Armstrong (3, 4) described severe subjective symptoms in man, and cerebral hemorrhages associated with rupture of posterior communicating branches of the circle of Willis in goats exposed to more than $-5\ g$. Jasper *et al.* (5) observed cerebral hemorrhage in 1 of 6 monkeys exposed to $-10\ g$ for periods of 10 to 20 seconds, but found no significant cerebral pathology in 6 cats exposed to $-4.5\ g$ for 30 seconds twenty times a day for 4 days. The carotid and cerebrospinal fluid pressures were both greatly increased during negative *g*.

Britton and Pertzoff (6) studied changes in carotid and femoral arterial pressures under both positive and negative *g*. They noted that the arterial pressure was increased promptly in the portion of the animal directed toward the periphery of the centrifuge. In the portion of the animal nearest the axis of rotation, the arterial pressure fell progressively, but not abruptly.

The following analysis of the pressure relationships within the cerebro-spinal cavity was used in designing the present experiments. The arterial blood pressure at neck level increases when the blood is forced in that direction under negative *g* (3–6). However, the cerebral vessels are surrounded by cerebrospinal fluid which is in turn contained within a semirigid cavity. A simple analogy

[1] This investigation was accomplished under contract N6ori77 Task #1 with the Office of Naval Research. Opinions expressed do not necessarily represent the views of the Navy.

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emphasizes the importance of this situation with regard to the protection which may be afforded the cerebral circulation under negative *g*. If a common rubber balloon is filled with water and fastened to a length of string, it will burst if rapidly whirled in a circle because the increased effective weight of the water increases the internal pressure in the balloon. Such a balloon submerged in a rigid container filled with water may be rotated at any speed because the pressure in the water inside the balloon is balanced by an equal pressure in the fluid on the outside of the balloon. The cerebral vessels may derive similar protection from the cerebrospinal fluid. Thus, if the increase in arterial, venous and capillary pressures were accompanied by an identical increase in cerebrospinal fluid pressure, the differential pressure supported by the vascular walls would be no greater under negative *g* than under normal conditions.

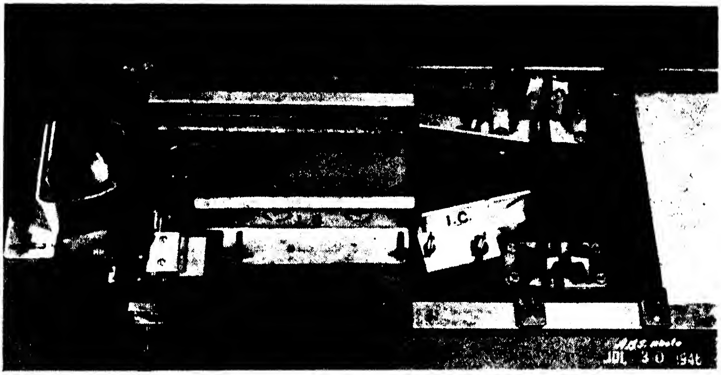

FIG. 1. Apparatus for restraining animals during exposure to radial acceleration. H.C., head clamp; P.C., pelvic clamp; C.S.F. and V.P., strain gauge pressure recorders.

Conversely, under the influence of positive *g*, the arterial blood pressure at the head and neck becomes progressively reduced as the magnitude of the force is increased (3, 4, 6, 7, 8). If the intracranial pressure is similarly reduced, the tendency for collapse of the vessels would be opposed.

To investigate these pressure relationships, simultaneous recordings have been made of the venous and cerebrospinal fluid pressures at the vertex of the skull and at neck level under the influence of positive and negative radial acceleration, respectively. Measurements were also made of the changes in carotid and jugular pressures during exposure to negative *g*.

APPARATUS. The human centrifuge consists of a bridge-like superstructure and a heavy flywheel which rotate independently on a large central axis. When the flywheel is rotating at some predetermined rate, the superstructure may be rapidly rotated by clutching it to the flywheel so that within a few seconds they are rotating at the same rate.

An animal board was constructed which allowed skeletal fixation at the head and pelvis (fig. 1). A head clamp, covered with sponge rubber, impinged below the mandible without applying pressure to the cervical vessels (H.C.). Lateral

supports of heavy gauge sheet metal could be adjusted by means of slots and wing nuts. Pelvic fixation was accomplished by means of extruded dural angles impinging upon the crest of the ilium on each side and clamped rigidly to the base board. A horizontal bar pressed firmly against the ilium. Two strain gauge pressure transmitters[3] (± 6 p.s.i.) were securely fastened to the base board at the level of measurement of the pressures. The center of the gauge used for recording cerebrospinal fluid pressure was exactly 3 cm. nearer the head than the venous pressure gauge which required a correction of recorded pressures.

METHOD. In the present series of experiments, cats were studied while under surgical anesthesia induced by intraperitoneal injections of nembutal (17 mgm/kgm.). The first series of animals was exposed to negative *g*. A cannula was inserted into the external jugular vein and connected by clear vinyl tubing to the appropriate strain gauge which was completely filled with saline-heparin solution. A short beveled 15-gauge hypodermic needle was gently inserted into the cisterna magna, fine vinyl tubing was passed through the needle into the subarachnoid space and the needle was withdrawn leaving the catheter in place. The free end of the catheter was connected to the second strain-gauge transmitter by vinyl tubing with a larger internal diameter. The board with the animal in position was mounted on the centrifuge with the head toward the periphery at a distance of 17 feet from the axis of rotation. The long axis of the body of the animal was along a radius of the circle described by the centrifuge. This position was maintained during rotation of the centrifuge. The strain gauges were fitted with Cannon plugs connected through mercury troughs to Heiland galvanometers (type A) in the control room where changes in pressure and the magnitude and duration of the accelerative forces were recorded. Roentenograms of the animals were obtained before and during the runs on 8 x 10 X-ray plates in cardboard casettes beneath the animal. For this purpose a General Electric X-ray machine (model F, type 4) was mounted 38 inches above the animal board. To correct for parallax a centimeter scale with lead markers was adjusted to the mid-axillary line of the animal (fig. 1, CS).

Each animal was exposed to 2 runs of 15 seconds' duration at −2, −3, −4, −5, −6 g. The order of exposure to the different levels of *g* was deliberately randomized. (Some of the animals received an additional series of 10 runs under positive g.) A series of 30 exposures to negative *g* was obtained on 2 animals while recording cerebrospinal fluid pressure with the strain gauge mounted near heart level.

To expose animals to positive *g*, the animal board was rotated 180°, so that the head was directed toward the axis of the centrifuge. Under these conditions the portion of the external jugular vein between the cannula and the heart became collapsed and the records were of no significance. For this reason cerebrospinal fluid and venous pressures were recorded from two hollow metal plugs threaded into holes into the superior sagittal sinus and adjacent subarachnoid space midway between the vertex and the occipital protuberance. The strain-gauge pressure transmitters were mounted at the level of measurement.

[3] Manufactured by Statham Laboratories, Los Angeles, California.

Simultaneous records were obtained of carotid and external jugular pressures only under the influence of negative acceleration.

RESULTS. The appearance of typical records of cerebrospinal and venous pressures obtained at various levels during positive and negative radial acceleration are illustrated in figure 2. The fluctuations in the two pressures associated with respiration and pulse were synchronous.

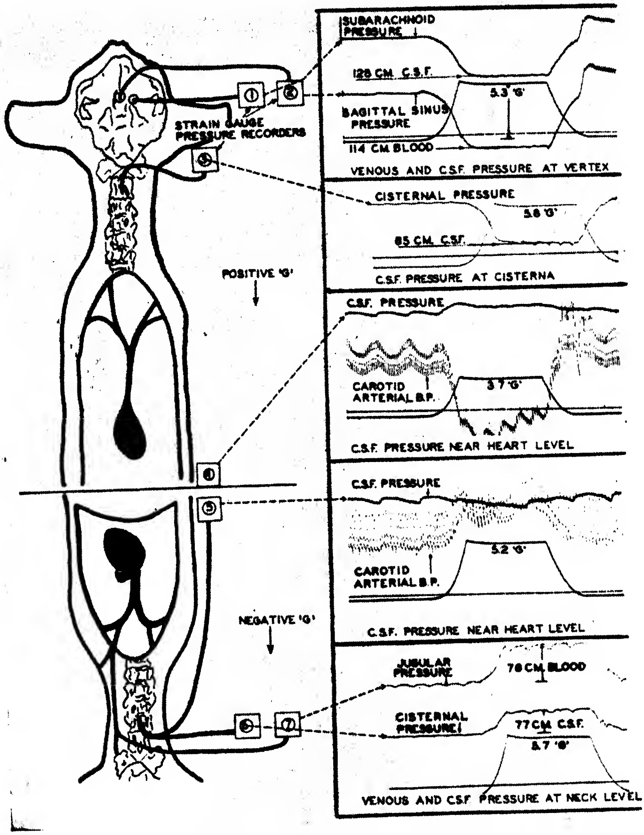

FIG. 2. Typical records of cerebrospinal fluid pressure and venous pressure recorded from various levels under positive and negative radial acceleration. The arrows indicate the direction of action of the accelerative force.

Recordings were obtained on 7 animals during 61 exposures to negative g, ranging from $-2\ g$ to $-6\ g$. Measurements on the records were made of the pressures sustained during the expiratory phases of respiration before and during acceleration. Venous and cerebrospinal fluid pressures increased by approximately the same amount (fig. 2, gauges 6, 7). To obtain a quantitative expression of the relationship between them, the increase in cerebrospinal fluid pressure was plotted against the corresponding change in venous pressure (fig. 3).

The coefficient of correlation (r) between these two variables was 0.91 and the regression coefficient (bxy) 0.93. Thus the C.S.F. pressure was directly proportional to venous pressure under the conditions obtaining in this experiment. Further, a unit increase in one variable tended to be associated with a unit change in the other. During positive radial acceleration the relation between the pressure in the superior sagittal sinus and adjacent subarachnoid space (fig.

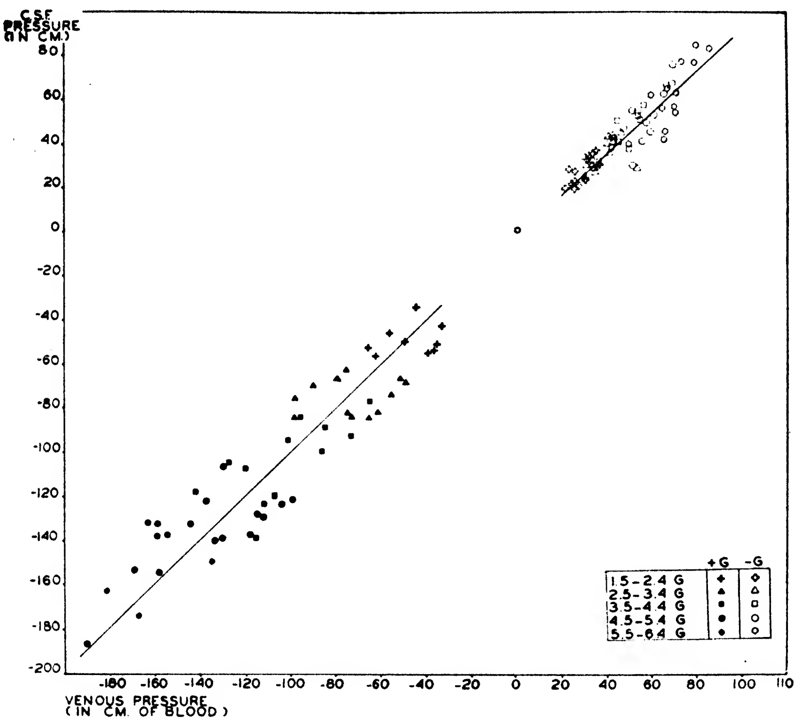

FIG. 3. Graphic illustration of the correlation of simultaneous measurements of cerebrospinal fluid pressure and venous pressure during positive and negative radial acceleration of varying magnitudes. Increased pressures occurred under negative g.

2, gauges 1, 2) is illustrated graphically in the lower left-hand portion of figure 3 ($r = 0.89$, $bxy = 1.00$). These correlations confirm the impression that C. S. F. and venous pressure are affected to about the same extent by positive and negative g.

Assuming that the observed pressure changes were caused by the influence of radial acceleration on hydrostatic columns of blood and cerebrospinal fluid, the heights of the columns of fluid involved may be calculated by dividing the changes in pressure by the magnitude of the force in g units.[4] For example, in

[4] The changes in venous and cerebrospinal fluid pressure have been expressed as cm. of blood and C.S.F. to facilitate these calculations.

figure 2 (gauge 6) the cerebrospinal fluid pressure increased by 77 cm. C.S.F. during a run at 5.7 *g*. Dividing 77 by 5.7 provides a quotient of 13.5 cm., representing a column of cerebrospinal fluid sufficient to produce the observed change in pressure. Measuring 13.5 cm. from the level of measurement at the neck toward the axis of rotation establishes the level at which the pressure was the same at the moment of measurement as it was during the control period. Determination of this level for each record of venous and C.S.F. pressure produced a distribution at or near heart level (fig. 4). The variability in the distribution was due to inability to measure the pressure changes at precisely the same instant the X-ray exposure was made. Changes in the position of the heart plus the augmented respiratory excursions contributed to the variability. Thirty determinations of cerebrospinal fluid pressure were obtained during exposure to

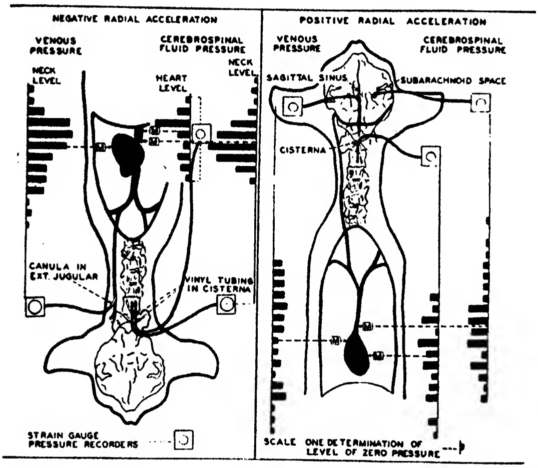

FIG. 4. Frequency distribution of computed levels at which the cerebrospinal fluid and venous pressures, at the moment the determinations were made, were the same during acceleration as during the control period.

negative *g* with the strain gauge slightly caudal to heart level (fig. 2, gauge 5). Under these conditions the recorded pressures decreased slightly during accelerations producing increased pressures at head level. The mean of the distribution was similar to those obtained at neck level (fig. 4).

During positive radial acceleration, the cerebrospinal fluid pressures in the cisterna magna and near the vertex of the skull, as well as the pressure in the superior sagittal sinus, were markedly decreased since the accelerative force was acting from head to tail. The height of a column of fluid capable of producing the observed reductions in the recorded pressure was calculated. Plotting the distribution of the computed levels at which the pressure was relatively unchanged during positive *g* reveals a great deal more variability than was noted with negative *g* (fig. 4). The mean values, nonetheless, were near heart level, and a few determinations of cerebrospinal fluid pressures, made with the strain

gauge near heart level, invariably revealed little change under positive g (fig. 2, gauge 4). It should be noted that the computed heights of the hydrostatic columns were considerably longer during positive g than under negative g. This is due to the fact that measurements were made at the vertex of the skull instead of at neck level, while the heart occupied a position lower in the chest during positive g.

Arterial and venous pressures were recorded from the carotid artery and external jugular vein in 7 animals during 68 exposures to negative g. Marked fluctuation in the arterial blood pressure was associated with respiration during the control periods (fig. 6). At the onset of the radial acceleration, the arterial

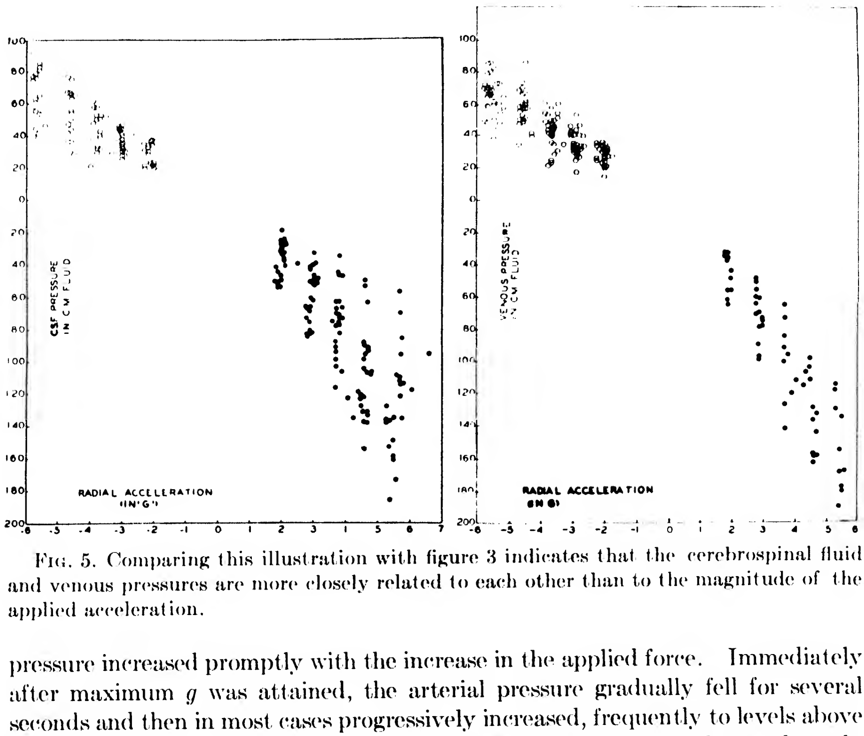

FIG. 5. Comparing this illustration with figure 3 indicates that the cerebrospinal fluid and venous pressures are more closely related to each other than to the magnitude of the applied acceleration.

pressure increased promptly with the increase in the applied force. Immediately after maximum g was attained, the arterial pressure gradually fell for several seconds and then in most cases progressively increased, frequently to levels above the initial level. The segment of the arterial pressure record which was abruptly depressed in the midportion of two of the records (fig. 6) was due to a drop in the line voltage supplying the strain gauges during the X-ray exposure.

In general, the fluctuations in arterial pressure due to respiratory activity during the application of negative g were synchronous with changes in venous pressure, but there were obvious differences in the amplitude of the deflections. The magnitude of the initial increase in arterial pressure was measured from an arbitrary base line, connecting the peaks of systolic pressure during the control period to the pressure attained immediately after maximum g was attained

(fig. 6). Since it was not possible to ascertain the phase of the respiratory cycle at the instant maximum g was attained, the recorded value may have been lower than the absolute increase in mean pressure by amounts as great as the respiratory fluctuations during the control period. Examination of the records revealed that the arterial and venous pressures increased by amounts roughly proportional to the applied negative g. This relationship is presented in graphic form in figure 6. The relation between the initial increase in arterial pressure and the sustained increase in venous pressure (r = 0.49) was not as close as

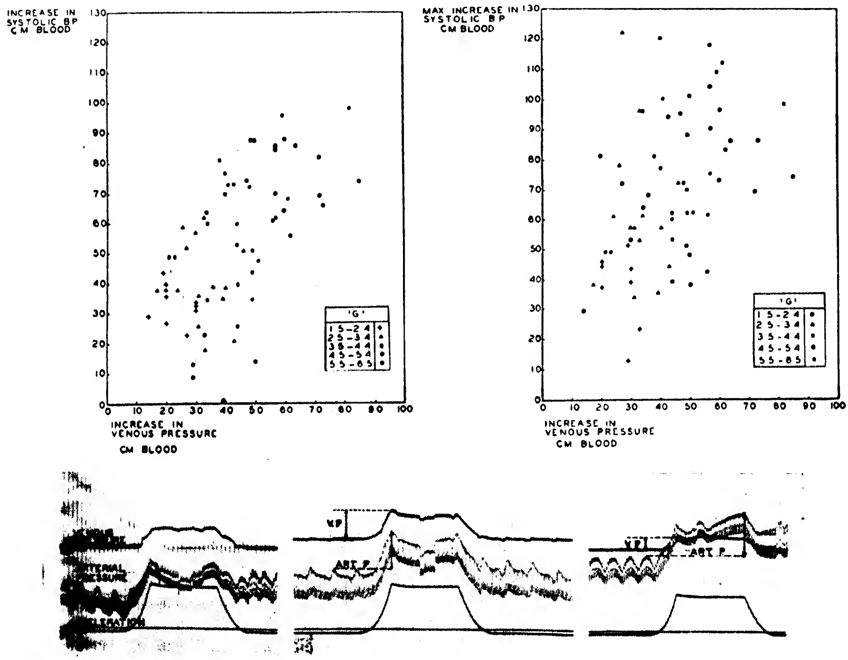

FIG. 6. Plotting the increase in carotid pressure against the increase in external jugular pressure reveals that the arterial pressure tended to increase more than the venous pressure at the same level of measurement. The secondary increase in arterial pressure is illustrated in the sample records.

previously described for cerebrospinal fluid and venous pressures. Plotting the maximum increase in arterial pressure (initial or secondary rise) against the change in venous pressure revealed that, in a majority of the determinations, the magnitude of the increase in arterial pressure was greater than the change in venous pressure. The maximum differential between the two pressures was 93 cm. of blood (61 mm. Hg).

DISCUSSION. The application of radial acceleration to experimental animals by means of the human centrifuge increases the effective mass of the body of the animal by predictable amounts. Under the influence of this type of force, the

pressure provided by a hydrostatic column of fluid of constant height is increased in direct proportion to the magnitude of the applied acceleration (in g units). In the series of experiments described above, anesthetized cats were mounted on the centrifuge in the horizontal position with the long axis of the body along a radius of the circle described by the centrifuge in motion. When the centrifuge was at rest, the force of gravity acted perpendicular to the long axis of the body and the venous and cerebrospinal fluid pressures were approximately equal at either end of the animal. During rotation of the centrifuge, the force of gravity continued to act in the same way, and, in addition, a force produced by the radial acceleration (centrifugal force) was directed along the radius of the circle toward the periphery. Under these conditions a gradient in hydrostatic pressure was produced along the long axis of the body of the animal such that high pressures were produced at the distal end of the animal with progressively lower pressures in the columns of fluid extending toward the axis of rotation of the centrifuge. Thus the cerebrospinal fluid and intravascular pressures, measured at neck level, increased under negative g (when the head of the animal was directed toward the periphery) and became subatmospheric at the vertex of the skull when the head of the animal was toward the axis of rotation (positive g).

By exposing the animals to various levels of radial acceleration extending from $+6\,g$ to $-6\,g$, cerebrospinal fluid pressures ranging from 80 cm. C.S.F. above normal to 190 cm. C.S.F. below normal were produced. Throughout this large range in pressures, the C.S.F. and venous pressures measured at the same level varied together and by approximately the same amount. In fact, the differences between them are no greater than might be explained on the basis of experimental error.

This was interpreted to indicate that the columns of C.S.F. and blood producing these pressure changes must be of approximately the same length. However, the subarachnoid space extends from the skull to the lowermost extremity of the dura mater in the lower spine. The fact that fluid can flow freely along the subarachnoid space is demonstrated during any lumbar puncture. Under these conditions, the hydrostatic column responsible for the changes in pressure observed during the application of radial acceleration might be expected to extend from the lower lumbar region to the level of measurement, at the neck or vertex. Further, there appears to be a continuous column of blood extending from the veins of the lower extremities through the right auricle to the jugular vein, or superior sagittal sinus. In this study it was possible to compute the extent of the effective hydrostatic column of fluid required to produce the observed alterations in pressure during exposure to g. It was found that, under the conditions obtaining during these experiments, both pressures fluctuated with respiration and pulse, but at heart level were relatively unaffected by longitudinal hydrostatic columns during exposure to either positive or negative radial acceleration. In the case of venous pressure, this could occur only if the right heart continued to expel all the blood coming to it through the vena cavae.

Because of the promptness of the response to respiration and g, it did not appear likely that the changes in cerebrospinal fluid pressure could be caused by

an alteration in the rate of formation or absorption of cerebrospinal fluid. A more plausible mechanism of the occurrence of such rapid responses in cerebrospinal fluid pressure is as follows. The tissues of the central nervous system, blood and cerebrospinal fluid may be considered virtually incompressible. They are enclosed in a semi-rigid container consisting of the skull, the vertebrae and their fibrous connections. Thus, a small increase in the total volume of the contents could produce a relatively large increase in pressure throughout the system. The fact that near heart level the changes in both the venous and cerebrospinal fluid pressure were small indicated that in all portions of the cerebrospinal cavity the cerebrospinal fluid pressure is nothing more than a reflection of the local venous pressure. In other words, at all levels within the cerebrospinal cavity, the venous and cerebrospinal fluid pressures are approximately equal, and, under normal conditions, they are probably in equilibrium with the extradural systemic venous pressure. There exist anastomoses between the veins within the dura and the extradural venous system at all levels from the vertex of the skull to the sacrum. These include the diploic, emissary, ophthalmic and jugular veins in the head and rich venous plexuses extending the whole length of the spinal column both inside and outside the vertebral canal, including the anterior and posterior longitudinal vertebral sinuses and the intervertebral veins (9). Under these conditions, so long as no pathologic changes occur, a difference in pressure between C.S.F. and venous pressure would result in expulsion or retention of blood within the venous reservoir or plexuses so that equilibrium could be maintained. Similarly the presence of numerous direct connections between the veins within the cerebrospinal cavity and the extradural veins should ordinarily prevent a differential pressure between the intradural and systemic veins. In this way the cerebrospinal fluid pressure should approximate extradural venous pressure at any level in the body so long as the veins of the central nervous system are neither collapsed nor distended to the point that their walls are under tension. This mechanism appears to explain the observations in the present study.

Further, if the differential between capillary pressure and venous pressure within the central nervous system were relatively constant, and venous pressure were in equilibrium with cerebrospinal fluid pressure, the difference between capillary pressure and C.S.F. pressure should be relatively constant regardless of the attitude of the body. In other words, the pressure supported by the capillary walls would be approximately the same at all points in the central nervous system. Such a condition should provide excellent protection for the circulation of the central nervous system, particularly the veins and minute vessels, in the presence of changes in posture and sudden changes in intravascular pressures. This may also be a contributing factor in maintaining very low concentrations of protein in the cerebrospinal fluid under normal conditions.

An increase in cerebrospinal fluid pressure would also tend to balance the augmented arterial blood pressure under the influence of negative g. The walls of the cerebral arteries would be required to support only the difference between these pressures. The pressures developed in the present study did not appear

excessive since the maximum differential was about 60 mm. Hg more than normal. It must be remembered, however, that in cats the hydrostatic columns from heart to brain are only about ½ to ⅓ the corresponding distances in humans. The present study does not rule out the possibility that hemorrhages originating in posterior communicating branches of the circle of Willis may result from an uncompensated increase in arterial pressure under negative g. However, an alternative possibility exists that mechanical tension may be applied to these vessels by extension of the head on the neck and elongation of the cervical spine. Under these conditions, traction on the vertebral arteries might be sufficient to encourage rupture of the posterior communicating arteries in the circle of Willis.

CONCLUSIONS

1. The cerebrospinal fluid and venous pressures measured at head and neck levels in anesthetized cats varied simultaneously and by approximately the same amounts during wide variations in these pressures produced by positive and negative radial acceleration.

2. Both C.S.F. and venous pressures remained relatively unchanged at or near heart level during exposure to either positive or negative *g*.

3. Under the conditions obtaining in these experiments, the cerebrospinal fluid pressure appears to be primarily a reflection of the venous pressure existing at any level within the cerebrospinal cavity.

4. The results of these studies indicate that the veins, and probably minute vessels, are almost perfectly protected against sudden changes in intravascular pressure by simultaneous changes of the same magnitude in cerebrospinal fluid pressure.

5. In a majority of cases, the arterial blood pressure at neck level increased by larger increments than the venous pressure at some time during the exposure to negative *g*. Since the arterial walls are called upon to support only the differential between intra-arterial and cerebrospinal fluid pressure, the cerebral arteries are afforded considerable but incomplete protection under these conditions.

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INCREASED SENSITIVITY OF HYPOTHERMIC RATS TO INJECTED POTASSIUM AND THE INFLUENCE OF CALCIUM, DIGITALIS AND GLUCOSE ON SURVIVAL[1]

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Received for publication September 26, 1947

When lightly anesthetized white rats are subjected to severe reduction of their body temperature, death occurs from circulatory and respiratory failure at body temperatures from 13° to 15°C. (1). In the course of studies of carbohydrate metabolism in cooled animals, the unexpected observation was made that animals starved for 24 hours survived to lower body temperatures than the fed controls (2). Preliminary investigation of this phenomenon showed that fed rats were able to survive body temperatures as low as those reached by starved animals if the fed animals were given calcium chloride intravenously or glucose in large doses by mouth.

The pattern of responses observed in the cooled rats included changes usually associated with vigorous activity of the sympathetic nervous system, namely, initial elevation of arterial pressure and heart rate (1) and sharp lowering of the glycogen content of the liver (2). Fenn (3) has presented evidence that the storage of glycogen in the liver also involves an increase in both hepatic water and potassium. When release of the previously stored carbohydrate occurs, there is a simultaneous release of water and potassium. Thus, during the induction of hypothermia in fed rats, the release of liver carbohydrate might be expected to be accompanied by addition to the circulating blood of both water and potassium. The first evidence that actions of potassium might play a part in the genesis of circulatory failure in cooled rats was indirect and depended upon the observations summarized below: *a*) animals with very low liver glycogen, e.g., animals starved 24 hours, survived to lower body temperatures than fed animals; *b*) animals starved for 48 hours had higher liver glycogen and died with higher body temperature than those starved for only 24 hours; *C*) the administration of glucose by mouth to rats both before and during cooling maintained the liver glycogen at relatively high levels throughout the period of reduced body temperature, and the animals survived to temperatures even lower than those reached by rats starved 24 hours (2); *d*) intravenous injection of calcium chloride solution extended the survival limits of fed rats to levels of body temperature as low as those observed in animals starved for 24 hours; *e*) injections of digitalis glycoside intravenously were as effective as glucose, starvation or calcium in reducing the lethal temperature limit (4).

[1] Supported by grants from the John and Mary R. Markle Foundation and from the Stanford University School of Medicine Research Fund.

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The study reported here presents quantitative data on changes in plasma potassium and calcium produced in rats by severe hypothermia and observations on the influence of hypothermia upon the threshold of cardiac response to injected potassium.

METHODS. Adult albino rats of the Slonaker-Wistar strain maintained at ordinary laboratory temperatures on a diet of Purina dog chow and tap water were cooled as described by Crismon (1). Body temperatures obtained from a thermocouple inserted into the rectum to a depth of 6 to 7 cm. were recorded on a Leeds and Northrup potentiometer. A Sanborn Stetho-Cardiette and Cardioscope were used for electrocardiographic observations during cooling. The usual three limb leads were employed.

Plasma potassium was determined by the method of Harington (5) modified to suit available equipment. Plasma calcium was determined by the method of Kramer and Tisdall (6) as modified by Clark and Collip (7).

RESULTS. Analysis of plasma from cooled rats indicated that the changes in calcium and potassium concentration during hypothermia were compatible with the hypothesis that a decrease in the calcium/potassium ratio might be a contributing factor to the circulatory collapse which occurs during cooling. Plasma potassium values were obtained on 14 normal rats and on 8 cooled rats. Samples from cooled rats were taken at a body temperature of 25°C. at which time shivering was vigorous. Since Brewer (8) showed that plasma potassium varied with metabolic activity and potassium is known to disappear rapidly from the blood stream (9, 10), it was felt that any rise in plasma potassium which might be attributed to hypothermia would be apparent at this point. The mean normal value was 3.44 ± 0.64 mEq/l., whereas the mean value for the plasma potassium of the cooled rats was 5.28 ± 0.70 mEq/l. Statistical analysis of the data by Fisher's method for comparing means of small groups (11) indicated that the difference was significant ($P < 0.01$). Total plasma calcium was determined on 5 normal rats and on 6 rats which had been cooled to 25°C. The mean value was 5.04 ± 0.46 mEq/l in normal rats and the mean value for the plasma from cooled rats was 5.72 ± 0.21. The difference between the means was significant ($P < 0.01$). Results of the analyses are included in table 1. Unfortunately, the biological method of McLean and Hastings (12) for the determination of ionic calcium proved unsatisfactory for use with rat plasma; hence no values for ionic plasma calcium were obtained. The total calcium/potassium ratios were reduced from 1.46 in normal uncooled rats to a value of 1.08 in rats cooled to 25°C.

Since the plasma potassium failed to rise to levels shown to be toxic (13, 14), the question arose as to whether or not hypothermia enhanced the response to changes in plasma potassium concentration. This problem was attacked in the following manner. An uncooled, anesthetized rat was given isotonic potassium chloride via the external jugular vein. The dose was adjusted to the calculated blood volume (7.4% of body weight) in amounts sufficient to double the potassium concentration if all of the added potassium remained in the blood. This procedure was repeated three times at 60-minute intervals. The injections

were completed in approximately 30 seconds, electrocardiograms being taken before, during and after each injection. Moderate cardiac irregularities which resembled those seen in typical potassium poisoning appeared during each injection, but the changes were reversible without evidence of persisting abnormalities.

However, a single injection of potassium chloride solution into rats cooled to 25–22°C. was fatal in each of 5 cases. The electrocardiographic changes resembled those of potassium poisoning reported by Winkler, Hoff and Smith (13) and those of clinical potassium poisoning reported by Keith *et al.* (15), by Finch and Marchand (16) and others. Complete cardiac arrest usually

TABLE 1. *Plasma calcium and potassium values for normal uncooled rats and untreated rats cooled to 25°C.*

CALCIUM—MILLIEQUIVALENTS PER LITER PLASMA		POTASSIUM—MILLIEQUIVALENTS PER LITER PLASMA	
Uncooled	Cooled	Uncooled	Cooled
5.46	5.76	3.22	4.13
4.91	5.74	3.17	4.13
5.18	5.23	3.41	5.17
4.91	6.19	3.38	5.29
4.82	5.83	2.46	4.71
		3.02	6.17
		4.17	6.40
		3.89	4.89
		3.10	5.48
		2.50	
		3.38	
		3.68	
		4.86	
		3.98	
$\bar{X} = 5.04$	$\bar{X} = 5.72$	$\bar{X} = 3.44$	$\bar{X} = 5.28$
$s_{\bar{x}} = 0.46$	$s_{\bar{x}} = 0.21$	$s_{\bar{x}} = 0.64$	$s_{\bar{x}} = 0.70$

Each value presents data from one rat.

occurred within 2 minutes after injection of the potassium chloride, respiration continuing about 5 minutes longer. These data indicate that amounts of potassium given by intravenous injection, which are well tolerated by rats at normal temperatures, produce fatal potassium poisoning in rats at reduced body temperature.

Direct evidence was obtained that glucose, calcium and cardiac glycoside will protect cooled rats against a fatal dose of potassium. Rats in groups of 3 animals each were subjected to the following procedures. One group was cooled and given 50% glucose by stomach tube approximately one hour before administration of the same dose of potassium given controls at 25°C. The potassium chloride was dissolved in 5% glucose solution. A second group was cooled and given intravenous calcium chloride about 25 minutes before the administra-

tion of isotonic potassium chloride at 25°C. A third group was given ouabain before cooling and isotonic potassium chloride approximately 150 minutes after 'digitalization' when the animals had been cooled to 25°C. Details of dosage, time relationships and fate of the animals are shown in table 2.

TABLE 2. *The effect of glucose, calcium and digitalis upon potassium toxicity in adult male rats cooled to 25°C.*

RAT	WEIGHT	INJ. TEMP.	PROTECTIVE TREATMENT	FURTHER EXPERIMENTATION
	grams			
4	230	25.0°C.	KCl injected in 5% glucose	5 cc. 50% glucose 18′ later followed by 0.081 mEq. K 50′ later. Cooled to 14.4°C. after recovery from second K injection.
5	204	25.6°C.	3 cc. 50% glucose 64′ before injection of KCl in 5% glucose	Cooled to 11.2°C.
6	232	25.4°C.	4 cc. 50% glucose 46′ before injection of KCl in 5% glucose	Cooled to 15.4°C. Died as result of aspiration of glucose solution.
7	228	24.5°C.	0.5 cc. of 0.11 M $CaCl_2$ I. V. 25′ before KCl injection	Cooled to 13.8°C and allowed to recover at room temperature.
9	192	26.8°C.	0.5 cc. of 0.11 M $CaCl_2$ I. V. 24′ before KCl injection	Cooled to 10.8°C. and allowed to recover at room temperature.
10	336	25.5°C.	1.0 cc. 0.11 M $CaCl_2$ I. V. 27′ before KCl injection	Cooled to 12.0°C. Died.
11	220	25.2°C.	Ouabain, 3 cat units/kgm. 151′ before KCl injection	Cooled to 10.4°C. Died
12	270	22.8°C.	Ouabain, 3 cat units/kgm. 151′ before KCl injection	Cooled to 10.4°C. Died.
17	230	25.0°C.	1/100 grain ouabain 160′ before KCl injection	Cooled to 21.0°C. and allowed to recover at room temperature

In sharp contrast to untreated animals given potassium chloride after being cooled to 25°C., all animals protected by the methods outlined above survived. Statistical analysis by Fisher's method for the exact treatment of 2 × 2 tables (11) indicated that the probability of chance occurrences of the observed results was only one in 2002 if the 3 groups were combined and compared with the

controls. When each group was compared with the controls separately, the probability of chance occurrence was one in 56.

Electrocardiographic changes, following potassium chloride injection into protected rats cooled to 25°C., were similar to those observed in uncooled controls. The responses of the heart to potassium were shown to be reversible by prompt restoration of normal cardiac cycles. The injection of the potassium chloride produced no permanent damage inasmuch as the animals survived to body temperatures lower than controls when cooling was continued after potassium injection. Two animals protected by calcium were allowed to recover from body temperatures of 13.8° and 10.8°C. and were in excellent condition 3 months later.

DISCUSSION. The changes in plasma potassium and calcium concentration in the blood of rats subjected to reduction of their body temperature are considerably below those which have been associated with unfavorable effects upon the hearts of mammals at normal body temperatures. The data of Winkler *et al.* (13) indicate that changes may be detected in the electrocardiogram when the plasma concentration of potassium is 5 to 7 mEq. per liter and that the changes become progressively more marked until diffuse block is noted at levels around 10 mEq. per liter and cardiac arrest at 14–16 mEq. per liter. Factors other than the absolute concentration in the plasma have been shown to have some influence on the response of the heart to potassium. If potassium concentration rises slowly, the plasma concentration reached at the time diffuse block first appears is higher than that noted when the rate of administration is rapid (14). The simultaneous administration of calcium ions with potassium permitted the elevation of potassium concentration to approximately 10 times the concentration found to have toxic action without protection by calcium (17).

Both potassium and calcium concentrations increase in the plasma of rats subjected to hypothermia, but the rise of calcium is much smaller than that of potassium, and the calcum to potassium ratio is substantially reduced. While no direct evidence is available on the plasma concentration of calcium in the ionized form under conditions of reduced body temperature, it is probably lower than at normal body temperature owing to the rise in plasma protein concentration which occurs during the induction of hypothermia. Thus the calcium effective for opposing potassium action on the heart would be actually less than the amount indicated by the concentration of total calcium measured.

The magnitude of the increase of potassium concentration in the plasma produced during the induction of hypothermia in rats is well within the amounts which might be anticipated under these conditions. Release of potassium from the liver during glycogen breakdown has been mentioned as a factor (2). The violent shivering, which persists well below the temperature level selected for blood sampling, should contribute some potassium to the blood (18), and the persistent peripheral vasoconstriction and relative anuria should interfere with its distribution to inactive tissues and its loss in the urine.

The extent to which an unfavorable calcium/potassium ratio may contribute toward cardiac failure and death from hypothermia is indicated by the effective-

ness of a variety of procedures, all of them in one way or another serving to prevent or correct the disturbed relationship of calcium and potassium. The contribution of potassium to the blood by the liver during cooling may be prevented by starting the induction of hypothermia after 24 hours' starvation when the liver is low in both gycogen and potassium or by enforcng the maintenance of high liver glycogen throughout cooling by the administration of large amounts of glucose (2). Calcium chloride injection during cooling effectively prevents an increase of the calcium/potassium ratio and permits survival to lower body temperatures. The cardiac glycosides probably exert part of their beneficial effect by their direct action on the ventricular muscle. However, it is proper to include them among the agents which may act on the heart in hypothermia by means of an influence upon calcium and potassium; potentiation of digitalis action by calcium (19, 20) and its inhibition by potassium (21) have been demonstrated.

The remarkable decrease in the tolerance of cooled rats to injected potassium lends support to the inferences drawn from the indirect evidence just presented. It is clear that the small increase of potassium concentration demonstrated in the plasma of hypothermic rats should not be interpreted in relation to concentrations shown to be toxic in animals at normal body temperature. Cold rats do not survive doses of potassium chloride which produce only transient changes in the electrocardiogram of uncooled controls, but present information does not permit precise description of the mechanism of the decreased tolerance. The added potassium may represent a sufficient increment upon the potassium entering the blood from the liver and skeletal muscle to produce lethal concentrations in the plasma; the rate of supply of calcium ions may be impaired at low temperatures; and there may be metabolic changes in the heart muscle which bring about a true change in sensitivity to potassium.

SUMMARY

1. Measurements of potassium and calcium concentrations in the plasma of rats with reduced body temperature showed small but statistically significant increases.

2. Plasma samples, taken when the body temperature was 25°C., contained 1.84 mEq/liter more potassium and 0.68 mEq/liter more calcium than the plasma of control rats at normal body temperature. The calcium/potassium ratios were reduced from 1.46 in normal animals to 1.08 in rats cooled to 25°C.

3. Intravenous injection of potassium chloride in amounts which produced only transient changes in the electrocardiogram of rats at normal body temperature produced fatal potassium poisoning in rats if administered when the body temperature was 22° to 25°C.

4. Injection of calcium chloride solution or ouabain and the administration of large doses of glucose by mouth, previously shown to lower the lethal temperature limits in rats subjected to hypothermia, also were found to protect the animals against doses of potassium chloride usually fatal at low body temperature.

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EFFECT OF PREGNANCY ON THE COURSE OF EXPERIMENTAL HYPERTENSION[1]

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Received for publication August 23, 1947

As a physiological variable complicating hypertension, a study of the effect of pregnancy on hypertension is of interest for the light it may throw on the probable nature of the fundamental defect in hypertension. Although hypertension is a relatively common disorder and is not infrequently a complication of pregnancy, observations on the human are contradictory and indefinite, due in part to the variability in the effects of pregnancy on the course of hypertension and in part to the fact that elevations in blood pressure accompany other complications of pregnancy which are unrelated to the hypertensive state. A study of the effect of pregnancy on the course of hypertension in the experimental animal was, therefore, undertaken in an attempt to elucidate the problem as it affects the human.

The present paper deals with the effects of pregnancy on the course of experimentally induced hypertension in the rat, rabbit and dog, the three species which have been most widely used in studies of renal hypertension in the laboratory. Several groups of investigators have reported their observations on the effects of pregnancy in these animals with diverse conclusions. Inasmuch as the hemodynamic factors as affected by pregnancy will differ in different species, it is important that any conclusion as to the fundamental effect of pregnancy on hypertension should be generally applicable since, insofar as our available data are concerned, experimental renal hypertension is fundamentally the same in all species.

METHODS. Hypertension was induced in rats, rabbits and dogs by applying a constricting figure-of-eight band to both kidneys or to one kidney with removal of the other. This procedure induces a chronic relatively constant elevation of blood pressure (1) and permits following the course of the disease over prolonged periods without the intervention of the complicating malignant phase of hypertension with a rapidly ascending level of blood pressure.

Blood pressures were determined in the rat by the plethysmographic method of Williams, Harrison and Grollman (2), in the rabbit by auscultation over the abdominal aorta as described by McGregor (3) and in the dog by puncture of the femoral artery and direct reading on a mercury manometer (4). In all of these

[1] Read before the Section on Obstetrics and Gynecology of the American Medical Association at its Centennial Session, Atlantic City, July 12, 1947.

[2] This study was aided by grants from The First Texas Chemical Company to the Department of Pharmacology, and from the John and Mary Markle Foundation for the study of hypertension.

procedures the blood pressure determinations are made on the unanesthetized animal. After a preliminary period of training, consistently constant results are obtainable from day to day.

Results. Since the effect of pregnancy on the level of the blood pressure in the hypertensive animal varies in different species, the data obtained on rats, rabbits and dogs will be presented separately.

Rats. The following observations are based on studies of 36 pregnancies which occurred in our colony of rats. As reported earlier (5) the elevated blood pressure of the hypertensive rat tends to fall during the course of pregnancy. This tendency of the blood pressure of the hypertensive rat to fall to relatively normal levels has been confirmed by all who have investigated this problem (6–8). The data obtained in the present study are summarized in table 1. In 18 of the 36 animals, i.e., in half of the animals, a pronounced decline in blood pressure occurred. This decline varied from 40 to 65 mm. (average 50 mm.). In about a third of the animals (11 of the total), however, the fall in pressure was

TABLE 1. *The average mean blood pressures of hypertensive rats during pregnancy*

The animals are grouped according to the degree of the observed decline in blood pressure.

NUMBER OF ANIMALS	AVERAGE MEAN BLOOD PRESSURE PRIOR TO PREGNANCY	AVERAGE MINIMAL MEAN BLOOD PRESSURE PRIOR TO DELIVERY	AVERAGE NUMBER OF YOUNG IN LITTER
	mm. Hg	*mm. Hg*	
18	175±10	120±7	9±2
11	178±12	160±10	6±2
6	176±10	172±8	4±1

only 12 to 23 mm. (average 18 mm.). In the remaining animals the change in pressure was minimal and within the range of the daily variation (10 mm.) in the blood pressure. Such a response was noted in 6 out of the 36 animals of the present series. In one animal only of the series was there a definite rise in blood pressure during the latter half of pregnancy.

The above-described findings again confirm the tendency of the blood pressure to fall during the latter part of pregnancy in the hypertensive rat. However, it must be emphasized that this is not a universal response, since in about one-sixth of the animals the fall in pressure is minimal or fails to occur and in an occasional animal an actual rise has been observed. The last-named response was noted only once in the present series and occurred in an animal which gave birth to a litter of only 3 young. The size of the litter apparently plays an important role in determining the response of the blood pressure in a given animal to a superimposed pregnancy. The average size of the litter in the 18 rats showing the maximal response (table 1) was 9; in the 11 animals whose response was slight, 6; while in the 6 rats showing little or no alteration in blood pressure an average of only 4 young were delivered per litter.

Pseudopregnancy induced by stimulation of the cervix with a glass rod results

in a reduction in the blood pressure of hypertensive rats, according to Page, Patton and Ogden (8). The induction of deciduomas by the insertion of silk threads into the uterine mucosa during pseudopregnancy, according to the authors, results in a reduction in the blood pressure comparable to that observed during pregnancy. These procedures have been repeated on a series of 6 animals but the average blood pressures observed prior and following the induction of the decidual reaction were essentially the same. Our results, therefore, do not support the contention that pseudopregnancy is accompanied by a reduction in blood pressure comparable to that seen during pregnancy.

Rabbit. The rabbit is particularly suited for the study of the effect of pregnancy on hypertension since it ovulates only with coitus and hence experimental studies may be planned better than with other species (9). The present conclusions are based on a study of 20 pregnancies and an equal number of pseudopregnancies in hypertensive rabbits. The latter were induced by mating with a male the vasa deferentia of which had been ligated to induce sterility.

TABLE 2. *The average systolic and diastolic blood pressures of hypertensive rabbits during pregnancy*

The animals are grouped according to the degree of the observed decline in blood pressure.

NUMBER OF ANIMALS	BLOOD PRESSURE PRIOR TO PREGNANCY		AVERAGE MINIMAL BLOOD PRESSURE ATTAINED PRIOR TO DELIVERY		AVERAGE NUMBER OF YOUNG IN LITTER
	Systolic	Diastolic	Systolic	Diastolic	
6	180±15	140±10	140±10	100±8	8±3
11	183±16	140±10	170±12	129±10	6±2
3	178±12	138±8	175±14	135±10	4±1

The effect of pregnancy on the level of the blood pressure of the hypertensive rabbit is essentially like that observed in the rat, except that the drop in blood pressure is not as marked nor does it occur with as great a frequency as in the smaller animal (10–12). Thus, of the 20 observed pregnancies, approximately half (11 instances) were accompanied by changes in pressure which were within the range of variation observed in the non-pregnant animal (table 2). In only 6 cases (about one-third of the total) were unquestionable reductions (40 mm.) in systolic and diastolic blood pressures observed. In the remaining 3 pregnancies no drop in pressure was observed.

Of the above-described 20 pregnancies, 4 occurred in the same animal, 3 in another and 2 in three others. In these instances of multiple pregnancies there was no consistency in the effect of the pregnancy on the level of the blood pressure in the same animal. As in the rat, the most important factor determining the response in a given pregnancy was the size of the litter, the observed drop in pressure being proportional to the number of young in the litter as shown in the last column of table 2.

Pseudopregnancy in the rabbit, as in the rat, was without a demonstrable effect on the blood pressure. The average of all the readings obtained during

the course of the 20 observed pseudopregnancies was less than 10 mm. below the average values observed prior to inducing pseudopregnancy. This slight reduction in the blood pressure is inconsequential as compared to the reductions observed during pregnancy and well within the experimental error.

Dog. Our observations on the dog have been limited to 5 pregnancies in 3 animals. The results obtained are in agreement with those of previous investigators (13, 14) and hence need only be summarized briefly. The reaction of the blood pressure in the dog is less uniform than in the smaller animal. In the majority of cases (3 of the 5 cases in the present series) there was a moderate drop in blood pressure during the latter half of pregnancy. In one case (in which only 3 pups were delivered) no change in blood pressure occurred. In the fifth case there was a gradual rise in the blood pressure during the first part of pregnancy from a mean pressure of 150 mm. prior to mating to 180 mm. one month later. This was followed by a gradual fall to 138 mm. observed prior to delivery. Following parturition the blood pressure returned during the course of 10 days to its original pre-gravid level.

The unmated dog normally undergoes a period of pseudopregnancy following estrus which lasts for 2 months and is accompanied by the usual changes of pregnancy (9). The blood pressure of hypertensive dogs during pseudopregnancy does not decline and indeed may rise slightly during this period.

COMMENT. The primary objects of the present study were to elucidate the pathogenesis of experimental hypertension and to apply the results to hypertension as observed in the human during pregnancy. No large series of observations are available on the changes in blood pressure induced by hypertension in the human (15–17). Such data as are available are in concordance with the present studies on the lower animals. The human reacts apparently in the manner to be anticipated from the comparative studies on the rat, rabbit and dog. As the size of the mammal increases, with a proportionate decrease in the relative size of the products of conception to the maternal mass, there is a diminishing effect of pregnancy on the level of the blood pressure in the hypertensive organism. In accord with this view are the observations that a reduction in blood pressure may at times occur in the hypertensive woman during pregnancy (17). Such a reduction is comparable to that observed in the experimental animal. However, as is to be anticipated from the comparative studies on the rat, rabbit and dog, this reduction is infrequent and in most instances little or no change is observed and in some cases the blood pressure may increase (18).

The possible deleterious effects of pregnancy on benign hypertension in the human has been a matter of controversy. In the experimental animal there is no evident deleterious effect of a pregnancy superimposed on the hypertensive animal. Deaths have not been observed during the present study in the pregnant or postparturient animal, despite the relatively high death rate in a hypertensive colony. This does not imply any protective action of pregnancy in hypertension since animals in an advanced stage of hypertensive cardio-vascular disease rarely become pregnant. Only those animals in a good state of health without the secondary complications of hypertension (cardiac hypertrophy,

uremia, anemia, etc.) tend to become pregnant and hence are capable of withstanding any slight deleterious effect which such superimposed pregnancy may induce on the underlying disorder. Recent clinical studies are in general agreement with this conclusion, namely, that the existence of hypertension itself is not a sufficient reason for terminating pregnancy and that the fetal death rate is not increased in the presence of essential hypertension (19).

As to the bearing of the present study on the pathogenesis of experimental hypertension, it would appear that the reduction in blood pressure observed at times during pregnancy is a result primarily of the increased size of the vascular bed induced by the presence of the placenta. This would account for the observation that the effect is less striking as one proceeds from the rat to the rabbit to the dog to the human. It would also explain the relation of the observed drop in any one species to the size of the litter. On the other hand one cannot exclude the possibility that the fetal kidney contributes something to the maternal organism which is responsible for the observed lowering of the blood pressure. This assumption would not be incompatible with the observed differences in different species, nor is it contradicted by the fact that the blood pressure during pregnancy in the normal animal is accompanied by no striking decrease in blood pressure. It has been demonstrated, for example, that certain procedures reduce the blood pressure in the hypertensive which are without effect on the blood pressure in the normal (20). It is true that the claim that removal of the embryos leaving the placentae intact does not interfere with the observed drop in blood pressure in the rat (21) would render the above-mentioned hypothesis untenable. However, it is difficult to interpret such experiments since the effect of operative shock involved in removing the embryos might account for the observed drop in blood pressure.

The fact that pseudopregnancy as shown in the present study is not accompanied in any species by an appreciable reduction in blood pressure would exclude hormonal factors as being concerned fundamentally in the observed drop in blood pressure during pregnancy.

It is clear from recent studies (15, 18, 22) that the rise in blood pressure which occurs in eclampsia cannot be considered as a manifestation of an underlying hypertensive diathesis. Contrary to earlier views, chronic hypertension is not a result of the presence in the circulation of a pressor agent (renin, angiotonin, or hypertension) (20, 23). On the other hand, the presence of such an agent has been demonstrated in eclampsia (7, 15), and the acute rise of blood pressure observed in this condition may be attributable to the elaboration by the ischemic kidney of this pressor agent. In none of our pregnant hypertensive animals have we observed the appearance of symptoms suggestive of eclampsia nor an increased tendency to abortion and fetal death, as claimed by Dill and his collaborators (24). Dawson, Cressman and Blalock (25) also were unable to confirm the claims of Dill and Erickson (24) as to the similarity of the hepatic lesions in hypertensive pregnant dogs to those observed in the human eclamptic and conclude that the genesis of the two conditions is different. Heuverswyn (12) was also unable to induce the eclamptic syndrome in rabbits by partial eschemia

of the kidneys of rabbits before or during pregnancy. On the other hand, in view of our present concepts as to the renal origin of hypertension (20, 30), it is understandable how the toxemias of pregnancy by their adverse affects on the kidney might induce permanent hypertension as a sequel to an initially extra-renal disturbance.

SUMMARY

A study has been made of the effect of pregnancy on the level of the blood pressure in hypertensive rats, rabbits and dogs.

The tendency for the blood pressure to decline during pregnancy in the hypertensive animal decreases with increasing size of the species, being less in the rabbit than in the rat, less in the former than in the dog and only occasionally observed in the human.

Pseudopregnancy as induced in the rat by stimulation of the cervix and the formation of a deciduoma, in the rabbit by sterile mating, and in the dog, as it occurs spontaneously following estrus, is not accompanied by any appreciable change in the blood pressure of the hypertensive animal. It is, therefore, unlikely that the hormones elaborated during pregnancy contribute to the observed changes. These are either secondary to the hemodynamic factors or depend upon the presence of the embryonic kidney.

The bearing of the present observations on the problem of pregnancy in the human complicating essential hypertension and the significance of the rise in blood pressure observed in eclampsia are discussed.

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EFFECT OF DILUTE SALINE SOLUTIONS ON THE GASTRIC POTENTIAL AND THE SECRETION OF HCl

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Received for publication September 30, 1947

In a series of experiments an attempt is being made to find out if the production of electrical energy by the stomach plays a rôle in the formation and secretion of HCl. One approach to this problem is a study of the relationship between the potential difference across the stomach wall and the secretion of HCl. In previous studies it was shown that, under certain experimental conditions, there was a correlation between the potential difference across the secreting stomach and the rate of secretion of HCl. The potential difference across the secreting stomach is approximately 40 mv. (the serosal side being positive in the external circuit to the mucosal side), and it was found that a depression of this potential difference was associated with a concomitant decrease in the rate of secretion of HCl. The methods used to depress the potential were *a*) application of relatively hypertonic hydrochloric acid solutions to the mucosa (2), *b*) application of a direct electric current from the mucosa to the serosa (3), and *c*) the application of ethyl alcohol-saline solutions to the mucosa (5). After withdrawal of these agents the secretory rate and the potential difference remained depressed for a matter of minutes and in most cases gradually returned over the course of about one hour to approximately their former magnitudes. The secretory rate never recovered without a concomitant increase in the potential difference.

The method used for making electrical contact in the above-described experiments was one in which 0.9% saline was placed in contact with the mucosal side of the stomach and 0.9% saline agar with the serosal side. Saturated KCl was used to connect the saline and saline agar with nonpolarizable electrodes (see fig. 3B). The potential difference (referred to in this paper as the over-all potential) measured in this way would be a function of both the potential of the stomach proper and the diffusion potential at the junction of the gastric secretion and saline.

The present paper is concerned with an apparent exception to the above-described relationship between the over-all potential and the rate of secretion of HCl. It was found that, when the 0.9% saline in contact with the mucosa was replaced with a relatively dilute saline solution, there was a definite decrease in the potential difference while the secretory rate did not show a significant change. An attempt has been made in the present paper to demonstrate that the decrease in the over-all potential in this instance is due primarily to an increase in the diffusion potential between the gastric secretion and the saline on the mucosal side and not to a change in the electrical characteristics of the stomach itself.

METHODS. The technique for measuring gastric secretion and potential as

described in a previous paper (2) was employed. With this technique a portion of the stomach along the greater curvature is placed between a ring of lucite and a lucite chamber. The ring of lucite has an oblique cut in it so that the blood vessels can be slipped inside it, thereby insuring an intact blood supply to the portion of the stomach in the chamber. The mucosal side of the stomach was oriented toward the chamber and the chamber was filled with 0.9% saline unless otherwise specified. The secretory rate was determined every 10 minutes by draining and flushing the chamber with 0.9% saline or one of the dilute salines. The total volume of each 10-minute sample was 100 ml. The pH and titratable acidity were determined on each sample in most of the experiments. In some experiments only the pH was measured. A nonpolarizable electrode made contact with the fluid in the chamber, and another similar electrode was placed in contact with the serosa. The potential difference was measured with a potentiometer.

Dogs were used in these experiments and sodium amytal (70 to 90 mgm. per kgm. subcutaneously) was used as the anesthetic agent. Histamine diphosphate was used to stimulate gastric secretion in all of the experiments and was administered subcutaneously at 10-minute intervals, usually in 0.5 mgm. doses.

It was found in all of the following experiments that the potential had the same orientation as that found in previous experiments, i.e., the serosal electrode was positive to the mucosal electrode in the external circuit.

RESULTS. *Effect of dilute saline solutions on the over-all potential and the rate of secretion of HCl.* Figure 1A represents a typical experiment in which 0.9% saline is replaced with 0.09% saline both before and after the secretory rate had reached a relatively constant level. It can be seen that there is a definite and immediate decrease of the over-all potential following the replacement. It is also to be noted that when 0.09% saline is replaced with 0.9% saline the over-all potential immediately returns to approximately its former value. In comparing the two 10-minute periods between 50 and 60 minutes and between 60 and 70 minutes in figure 1A, when 0.9% saline was in the chamber, it can be seen that the potential-time curve of the first period following the use of 0.09% saline is practically identical with that of the second period. However, it should be pointed out that the first few readings, when 0.09% saline is replaced with 0.9% saline, were invariably about one to three millivolts less than those when 0.9% saline was replaced with more 0.9% saline.

It was found that replacement of 0.9% saline with 0.09% saline did not have any significant effect on the secretory rate. This is illustrated in figure 1A and 1B. Histamine injections were started 39 minutes before the first values shown in figure 1A and 18 minutes before those shown in figure 1B. The increase in secretory rate was quite typical of that found in experiments in which 0.9% saline was used throughout. It was also found (illustrated in fig. 1) that, once the secretory rate leveled off, the use of dilute salines was not associated with a significant change in the secretory rate.

Replacement of 0.9% saline with salines intermediate in concentration between 0.9% and 0.09% resulted in a decrease of the over-all potential and no signifi-

cant change in the secretory rate. Figure 1B represents a typical experiment in which 0.09%, 0.36% and 0.63% saline solutions were used. It can be seen that the more dilute the saline the greater was the drop in the over-all potential. After replacement of 0.9% saline with a dilute saline, the over-all potential did not drop to its lowest value immediately, but reached its minimum value only after about 3 minutes (fig. 1A and 1B) and then gradually increased during the remainder of the 10-minute period. It was also found that the more dilute the saline the greater was the increase of the over-all potential from its minimum value to its value at the end of the 10-minute period.

In figure 1A it can be seen that the minimum value for the potential in 0.09% saline was lower during a second consecutive period in which 0.09% saline was

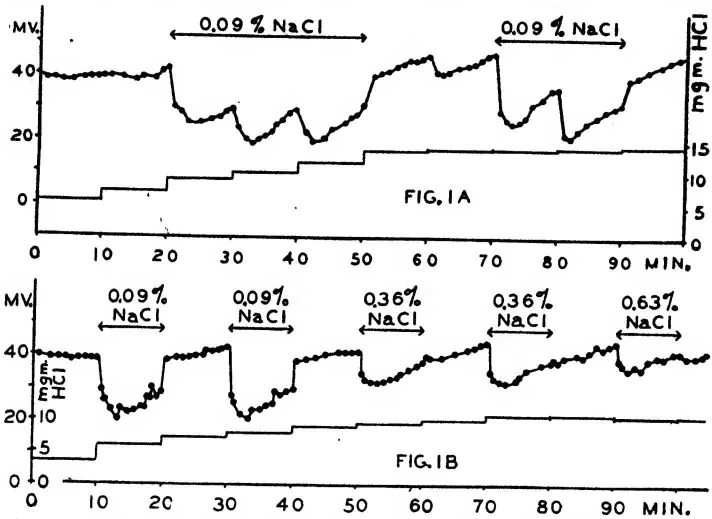

FIG. 1A AND B. Relationship between the potential difference across the stomach wall and the rate of secretion of HCl. The line connecting the solid dots represents the potential difference in millivolts. The other line represents the rate of secretion of HCl as milligrams of HCl secreted per 10 minutes. The fluid in the chamber was renewed every 10 minutes and 0.9 % saline was used unless otherwise specified.

used. Experiments were performed on 4 different dogs and this was found to be true in every case. The average decrease (minimum value in 0.9% saline minus the minimum value in 0.09% saline) in the over-all potential during the first period in which 0.09% saline had replaced 0.9% saline was 16 mv. with a range of values of from 14 mv. to 19 mv. The average decrease during a second consecutive period in which 0.09% saline was used was 21 mv. with a range of values of from 19 mv. to 23 mv.

Three experiments were performed (on 3 dogs) in which a series of dilute saline solutions were used (0.09%, 0.18%, 0.36%, and 0.63%). In these experiments the dilute saline solutions were placed in the chamber for a 10-minute period and then replaced with 0.9% saline. In figure 2 the decrease of the over-all poten-

tial (minimum value in 0.9% saline minus the minimum value in the dilute saline) is plotted for each experiment against the logarithm of the concentration times one hundred. It can be seen that the decrease in the potential is roughly proportional to the logarithm of the concentration of saline.

Magnitudes of the liquid junction potentials (diffusion potentials) between HCl solutions and saline solutions. As was pointed out previously (2, 5), there is in all probability a diffusion potential between the gastric secretion and the fluid in the chamber (see fig. 3B). The gastric secretion diffuses outward into the fluid of the chamber and hence this junction would be formed at a distance from

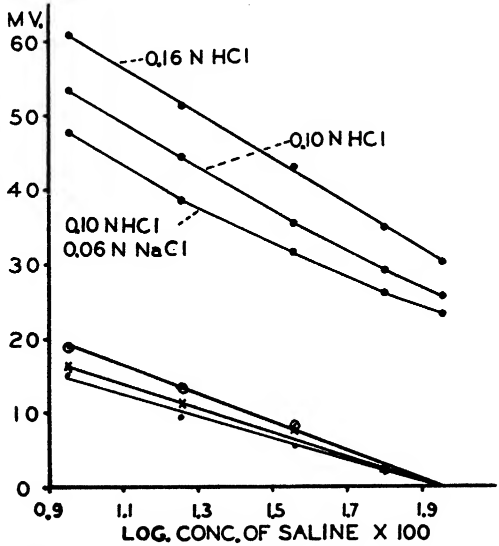

FIG. 2. In the three lines in the upper part of the figure the magnitudes of the diffusion potentials between the above-designated HCl solutions and a series of saline solutions (0.09%, 0.18%, 0.36%, 0.63% and 0.9%) are plotted against the logarithms of the saline solutions times one hundred.

The lower three lines represent the maximum decrease in millivolts in the magnitude of the over-all potential of the secreting stomach when 0.9% saline is replaced by a dilute saline solution (same series of saline solutions as used above in the diffusion potential studies).

the actual surface of the mucosa. Therefore, this junction would be in an aqueous medium and the orientation of its potential would obviously be such as to tend to make the mucosal electrode positive to the serosal electrode in the external circuit. Since the over-all potential (E_0 in fig. 3B) has the opposite orientation (the serosal electrode is positive in the external circuit), it follows that an increase in the magnitude of the diffusion potential would result in a decrease in the over-all potential. Therefore, a knowledge of the magnitudes of the diffusion potentials between a hydrochloric acid solution of a concentration comparable to that of the gastric secretion and the saline solutions would be necessary in evaluating the decrease in the over-all potential following the use of dilute

salines. It would probably be possible to obtain these data from scattered sources in the literature but, since the magnitude of diffusion potentials varies slightly with the method used for their establishment, it would be better for the purposes of comparison to determine the magnitudes of these diffusion potentials with one given method.

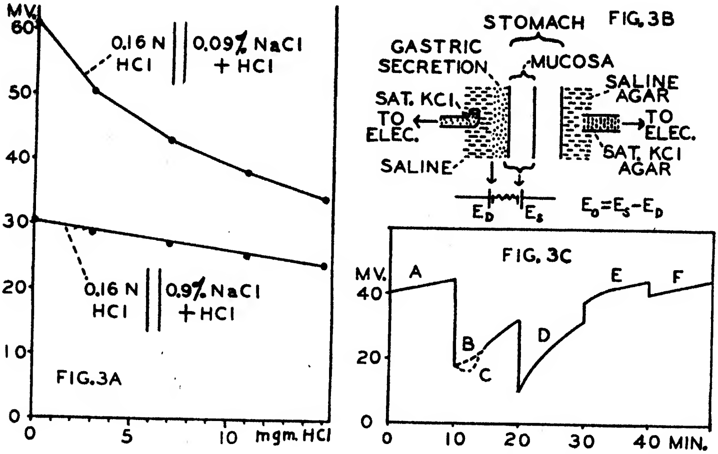

FIG. 3A. The top curve represents the magnitudes of the diffusion potentials in millivolts between a 0.16 N HCl solution and mixtures of 0.16 N HCl and 0.09% saline. The bottom line gives the magnitudes of the diffusion potentials between 0.16 N HCl and mixtures of 0.16 N HCl and 0.9% saline. The abscissa represents the number of milligrams of HCl added (as 0.16 N HCl) to 20 ml. of a given saline.

FIG. 3B. Schematic drawing showing the method for measuring the magnitude of the potential difference across the stomach. E_O represents the measured potential difference between the two electrodes and is referred to in the text as the over-all potential. E_D represents the magnitude of the diffusion potential between the gastric secretion and the fluid on the mucosal side. E_s represents the potential of the stomach proper (including potentials that might arise at the junction between the fluid on the mucosal side and the mucosa itself). See (4) for evidence that the E.M.F. (or E.M.F.'s) that gives rise to the stomach potential is located between the submucosa and fluid in contact with the mucosa.

FIG. 3C. Theoretical curves of the over-all potential (E_O of fig. 3B) constructed on the assumption that changes in the magnitude of the over-all potential are due entirely to changes in the magnitude of the diffusion potential. Curves A, E and F were constructed *for 0.9% saline, and B, C and D for 0.09% saline. In construction of these curves it is assumed that HCl is added to the fluid on the mucosal side at the rate of 1 mgm. of HCl per* minute. See text for further description.

The magnitudes of the diffusion potentials between three different HCl solutions and the saline solutions used in the above experiments were determined by the same method described before (5). In this method a given HCl solution was placed in a 'J' shaped glass tube with a diameter of approximately 4 mm. and this tube was lowered into a relatively large volume of a given saline solution. Saturated KCl solutions were used to connect these solutions to isoelectric,

in fig. 1 of a former paper (2)) rhythmically rises and falls). There are also rhythmic fluctuations in the outlet tube of the chamber that are correlated with the animal's respiratory movements. These fluctuations are probably due to a small displacement of the mucosal chamber by the respiratory movements. It is assumed that these rhythmic movements serve to mix effectively the secreted gastric juice with the fluid in the chamber. Therefore, since the HCl content of the chamber fluid increases with time, a knowledge of the magnitudes of the diffusion potentials between a hydrochloric acid solution comparable in concentration to gastric juice and mixtures of this hydrochloric acid solution and the salines would be necessary in attempting to interpret the changes in the magnitude of the over-all potential with time.

The volume of the fluid in the chamber varies somewhat, depending primarily on the position of the serosal electrode (see fig. 1 of a former paper (2)). However, the volume of the fluid in the chamber is in the neighborhood of 20 ml. (plus or minus a few ml.). The maximum secretory rate in the experiments presented in the present paper is in the neighborhood of 15 mgm. of HCl per 10 minutes. The magnitudes of the diffusion potentials between 0.16 N HCl and mixtures of 0.16 N HCl and saline solutions were determined by the method described above. The results are shown in figure 3A. The ordinates represent the diffusion potential in mv. between 0.16 N HCl and a given saline-HCl mixture. The abscissa represents the number of mgm. of HCl added (as 0.16 N HCl) to 20 ml. of a given saline solution. It can be seen that for both 0.9% and 0.09% saline the addition of HCl results in a decrease in the magnitude of the diffusion potential, and that the amount of decrease is greater when a given amount of HCl is added to 0.09% NaCl than when added to 0.9% NaCl. On the basis of these data one would expect the diffusion potential between the gastric juice and the fluid in the chamber to decrease with time, and, therefore, the over-all potential to increase with time. It will be recalled that the over-all potential decreases during approximately the first 3 minutes and then increases during the last 7 minutes. Since the over-all potential decreases during the first 3 minutes, it is probable that some other factor or factors, in addition to the diffusion potential, must be operating. On the other hand, it is probable that the increase in the over-all potential during the last 7 minutes is due in part to a decrease in the diffusion potential caused by the gradual addition of gastric juice to the fluid in the chamber.

The data presented in figure 3A would lead one to expect, for a given secretory rate, a greater increase in the over-all potential with time when 0.09% saline is used than when 0.9% saline is used. It was found in every experiment that the increase in the potential from its minimum value to its maximum value at the end of a 10-minute period was greater in 0.09% than in 0.9% saline.

Magnitudes of the diffusion potentials between 0.16 N HCl and mixtures of 0.16 N HCl and saline solutions and between these latter solutions and saline solutions. When the chamber is drained and flushed with saline it is possible that there is a layer of a mixture of gastric juice and saline near the mucosal surface that is not washed out by this procedure. If this is the case, then when 0.9% saline is

nonpolarizable electrodes. The magnitude of a given diffusion potential reached a relatively constant value within 4 minutes and, for purposes of comparison, the magnitude of a diffusion potential was taken as its value 4 minutes after its establishment. The first reading was made approximately one minute after the establishment of the junction and its value was within 2 mv. of the final reading. At least two determinations were made for each diffusion potential and the results in this and the following sections are the averages of these readings. Three different HCl solutions were used: *a*) 0.16 N HCl, *b*) 0.10 N HCl and *c*) 0.10 N HCl plus 0.06 N NaCl (1). These experiments were carried out at room temperature (approximately 23°C.). Although the temperature of the fluids in the chamber was approximately 39°C., the difference between the diffusion potentials at the two temperatures, on the basis of theoretical considerations, would be unimportant from the point of view of these experiments. The results of these measurements are shown in figure 2. The magnitudes of the diffusion potentials are plotted against the logarithm of the concentration of saline times one hundred. It can be seen that the diffusion potential between 0.16 N HCl and 0.9% saline is approximately 30 mv., while that between 0.16 N HCl and 0.09% saline is approximately 61 mv. Therefore, the magnitude of the diffusion potential between 0.16 N HCl and saline is increased by approximately 31 mv. when 0.09% saline is substituted for 0.9% saline. Similarly, the magnitude of the diffusion potential between the solution containing 0.10 N HCl plus 0.06 N NaCl and saline is increased by approximately 25 mv., and that between 0.10 N HCl and saline by 28 mv., when 0.9% saline is replaced by 0.09% saline.

It should be pointed out here that the small amounts of ions present in gastric juice other than H^+, Na^+, and Cl^- would not be expected to influence materially the magnitude of the diffusion potential. This was confirmed by the finding that the magnitude of the diffusion potential between an artificial gastric juice (made according to the formula of Rosemann (6)) and an isotonic solution of the same H^+ concentration composed of HCl and NaCl was practically zero (less than 1 mv.).

Assuming that the concentration of HCl in the fluid being secreted by the stomach is somewhere between 0.10 N HCl and 0.16 N HCl, one would expect, on the basis of the above measurements, a reduction of the over-all potential when 0.9% saline is replaced with 0.09% saline of from 25 to 31 mv. From a quantitative standpoint the actual decrease in the magnitude of the over-all potential is, therefore, less than the expected change from the *in vitro* measurements. There are other factors, however, that probably influence the magnitude of the diffusion potential (E_D, fig. 3B), and in the following sections an attempt is made to analyze in more detail the effects of changing the composition of the chamber fluid on the over-all potential.

Measurement of the diffusion potentials between 0.16 N HCl and mixtures of 0.16 N HCl and saline solutions. During the secretion of gastric juice the HCl content of the chamber fluid is being constantly increased. The stomach in the chamber usually undergoes rhythmic contractions at a rate of approximately three complete cycles per minute (the fluid in the outlet tube of the chamber (T_2

replaced by 0.09% saline, there would be initially a thin layer of a mixture of 0.9% saline and gastric juice near the mucosal surface, while the body of fluid in the chamber would be 0.09% saline.

Theoretical considerations would lead one to expect the interposition of a layer of a mixture of 0.9% saline and gastric juice between gastric juice and 0.09% saline to decrease the magnitude of the diffusion potential. An attempt was made to obtain some idea of this effect by measuring the magnitudes of the diffusion potentials between 0.16 N HCl and a half-and-half mixture of 0.16 N HCl and 0.9% saline, and between this latter solution and 0.09% saline.

It was found that the potential difference between 0.16 N HCl and a solution containing 0.08 N HCl and 0.45% NaCl was 10.5 mv., and the potential difference between this latter solution and 0.09% NaCl was 41.5 mv. Therefore, the potential difference between 0.16 N HCl and 0.09% NaCl with a solution containing 0.08 N HCl and 0.45% NaCl interposed would be equal to 52.0 mv., i.e., 10.5 mv. plus 41.5 mv. The potential difference between 0.16 N HCl and a mixture of 0.08 N HCl and 0.045% NaCl was 12.0 mv., and the potential difference between the latter solution and 0.09% NaCl was 47.5 mv. Therefore, the potential difference between 0.16 N HCl and 0.09% NaCl with a solution containing 0.08 N HCl and 0.045% NaCl interposed would be equal to 59.5 mv., i.e., 12.0 mv. plus 47.5 mv.

Therefore, granting the above assumption, one would expect that initially the over-all potential would be higher when 0.9% saline is replaced with 0.09% saline than when 0.09% saline is replaced with more 0.09% saline. This was actually found to be the case. It will be recalled that the minimum value of the over-all potential was greater (by 5 mv., i.e., 21 mv. minus 16 mv., see above) when 0.9% saline was replaced with 0.09% saline than when 0.09% saline was replaced with more 0.09% saline. It was also found that the initial value of the over-all potential was greater in every case when 0.9% saline was replaced by 0.09% saline than when 0.09% saline was replaced by more 0.09% saline. The average value for this difference was 7.4 mv. with a range of values from 6.3 mv. to 8.8 mv. (a total of six experiments).

A somewhat comparable situation would be expected to exist when 0.09% saline is replaced with 0.9% saline. Under these conditions the situation would be one in which a mixture of 0.09% saline and gastric juice would be initially present near the mucosal surface. An attempt was made to obtain some idea of the influence of the interposition of this layer on the magnitude of the diffusion potential between gastric juice and 0.9% saline by measuring the diffusion potentials between 0.16 N HCl and a half-and-half mixture of 0.16 N HCl and 0.09% saline and between the latter solution and 0.9% saline. It was found that with a half-and-half mixture of 0.16 N HCl and 0.9% NaCl interposed between 0.16 N HCl and 0.9% NaCl, the potential difference was 29 mv. (10.5 mv. plus 18.5 mv.), while the interposition of a half-and-half mixture of 0.16 N HCl and 0.09% saline gave a potential difference of 34 mv. (12.0 mv. plus 22 mv.). Therefore, when 0.09% saline is replaced with 0.9% saline, one would expect the over-all potential to be initially a few millivolts less than when 0.9% saline is re-

placed with more 0.9% saline. It was found in every case that the initial readings, when 0.09% saline is replaced with 0.9% saline, were lower than when 0.9% saline was replaced by more 0.9% saline. The average difference was approximately 2 mv. (range of values 1 mv. to 3.5 mv.).

DISCUSSION. On the basis of the foregoing observations on the diffusion potentials, theoretical potential-time curves were constructed on the assumption that the potential of the stomach proper (E_s in fig. 3B) remains constant and that all the changes in the over all potential are due to changes in the diffusion potential (E_D in fig. 3B). The curves shown in figure 3C were constructed for a secretory rate of 10 mgm. of HCl per 10 minutes, assuming the rate of secretion remains constant. From the data in figure 2 the diffusion potential, when 0.9% saline is replaced by more 0.9% saline, would be expected to be at its maximum value (approximately 30 mv.) immediately after the replacement, and then decrease over the course of a 10-minute period at an approximately linear rate to 26 mv., i.e., after 10 mgm. of HCl had been secreted into the chamber. The over-all potential would, therefore, be expected to increase in a linear fashion by approximately 4 mv. during a 10-minute period (curves A and F in fig. 3C). Similarly, when 0.09% saline was replaced by more 0.09% saline, the diffusion potential would be expected to be at its maximum value (approximately 61 mv., 31 mv. greater than with 0.9% saline) immediately after the replacement and then decrease over the course of a 10-minute period to approximately 39 mv. (a decrease of about 22 mv.). The over-all potential would, therefore, be expected under these conditions to be initially 31 mv. less than with 0.9% saline and then to increase in the manner shown in curve D of figure 3C by approximately 22 mv. The initial decrease in the over-all potential would be approximately 7.5 mv. less (59.5 mv. minus 52 mv.) when 0.9% is replaced by 0.09%, than when 0.09% saline is replaced by more 0.09% saline. It will be recalled that the assumptions were made that a layer of a mixture of 0.9% saline and gastric juice was initially present after the replacement of 0.9% saline with 0.09% saline. The actual shape of the curve during the first period in 0.09% saline would be determined by the rate of addition of gastric juice to the fluid and also by the time taken to replace the 0.9% saline-gastric juice layer. It is assumed in the construction of the theoretical curves in figure 3C that this 0.9% saline-gastric juice layer is replaced within approximately 5 minutes. The over-all potential curve could, therefore, during the first 5 minutes, gradually increase with time, or it could temporarily decrease below the initial value. This latter possibility would be realized if the 0.9% saline-gastric juice layer was rapidly replaced. These two possibilities are illustrated in curves B and C of figure 3C. When 0.09% saline is replaced with 0.9% saline, the over-all potential would be expected to be initially a few millivolts less than when 0.9% saline is replaced by 0.9% saline (compare initial values of E and F in figure 3C).

On the basis of the above analysis, it can be seen that most of the characteristics of the actual over-all potential curves can be explained on the assumption that the changes are due to diffusion potential changes and that the potential of the stomach itself (E_s of fig. 3B) does not change. However, the possibility is not ruled out that some portion of these changes in the over-all potential

may be due to changes in the potential of the stomach itself. For example, it is possible that a membrane potential exists at the surface of the mucosa which would be influenced by variations in the composition of the fluid coming in contact with it, and that the composition of this fluid would be partially dependent on the composition of the fluid in the chamber.

The one aspect of the over-all potential curves that has not been explained, when dilute saline replaces 0.9% saline, is the finding that the over-all potential does not drop initially to its minimum value but continues to decrease from its initial value for approximately 3 minutes. In this connection it is pertinent to note that a decrease of the over-all potential during the first few minutes after draining and flushing frequently occurs in the resting stomach when no HCl is being secreted. However, in the resting stomach this decrease, which is followed by a corresponding increase, is usually less than 3 mv. and in the great majority of cases less than 4 mv. It is reasonable to conclude, therefore, that in the secreting stomach this decrease during the first few minutes is at least partly due to factors other than the change in the magnitude of the diffusion potential between gastric juice and the fluid in the chamber. A factor that might conceivably play a part in this decrease during the first few minutes is the presence of a diffusion potential at the end of the tube containing the saturated KCl. The fluid in the chamber from the previous 10-minute period might have replaced the KCl solution at the tip of the KCl tube, which would probably result in the establishment of a diffusion potential oriented in the same direction as the over-all potential. This factor was ruled out on the basis of the following observations. It was found that flushing the KCl junction did not essentially change the character of the curves. After flushing this junction with saturated KCl (approximately 0.01 ml. flushed through at a time) the over-all potential, especially with dilute saline in the chamber, would in most cases increase by a few millivolts and then decrease. Except for this temporary increase following the flushing with KCl, the results of experiments in which this junction was not flushed during a given 10-minute period are quantitatively similar to those in which it was flushed several times.

Comparison of the magnitude of the decrease of the over-all potential at the end of 3 minutes, after 0.9% saline is replaced with 0.09% saline, with the expected decrease from the data presented in figure 3A reveals that there is a relatively good agreement between these two quantities. For example, if one assumes that 3 mgm. of HCl have been added to the fluid in the chamber by the end of 3 minutes, then from the data in figure 3A it can be seen that the expected increase of the diffusion potential, when 0.9% saline is replaced with 0.09% saline, is approximately 22 mv. (50.5 mv. minus 28.5 mv.). It will be recalled that the average decrease in the over-all potential (determined at approximately the end of 3 minutes) during a second consecutive period with 0.09% saline in the chamber, when compared to the value when 0.9% saline was in the chamber, was 21 mv. Since this agreement is relatively good, it is reasonable to believe that the actual magnitude of the diffusion potential at the end of 3 minutes is (from fig. 3A) approximately 50 mv. when 0.09% saline is in the chamber, and therefore the magnitude of the gastric potential itself (E_s of fig.

3B) would be equal to $E_0 + E_D$ (20 mv. + 50 mv.), or 70 mv. Similarly, the actual magnitude of the diffusion potential at the end of 3 minutes, when 0.9% saline is in the chamber, would be 28.5 mv., and therefore the magnitude of the gastric potential under these conditions would be approximately equal to 40 mv. + 28.5 mv., or 68.5 mv.

In previous work (2, 3, 5) in which the application of certain agents to the stomach resulted in a decrease in both the secretory rate and the over-all potential, it was found that after withdrawal of these agents the over-all potential did not return immediately to its original level, but only gradually returned, usually over the course of about one hour. The fact that the over-all potential immediately returns to approximately its original value when a dilute saline solution is replaced by 0.9% saline, considered in the light of the findings of the previous work, constitutes convincing evidence that the decrease in the over-all potential with the dilute saline is essentially due to a change in the magnitude of the diffusion potential.

The present work and the previously reported work, taken together, offer convincing evidence in support of the hypothesis that a decrease in the potential of the stomach itself (E_s of fig. 3B) is associated with a concomitant reduction of the rate of secretion of HCl.

It is evident from the present work that in studies of the potential difference across the stomach, it is important not only to eliminate the liquid junction potential between the mucosal electrode and the fluid in contact with the stomach, but also to know the composition of this fluid.

SUMMARY

Replacement of 0.9% saline solution in contact with the mucosa of the secreting stomach by a more dilute saline solution resulted in a decrease in the potential difference across the stomach and no significant change in the rate of secretion of HCl. A detailed study of the magnitudes of the diffusion potentials between HCl solutions, comparable in concentration to gastric juice and saline solutions, was made in an attempt to demonstrate that the decrease in the potential difference across the secreting stomach, when dilute saline solutions replaced 0.9% saline solution, is due to an increase in the diffusion potential between the gastric secretion and the fluid in contact with it. It is concluded, as the result of an analysis of these findings, that the decrease in the potential difference across the secreting stomach is due primarily to an increase in the diffusion potential and not to a change in electrical characteristics of the stomach itself.

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ROLE OF DIETARY PROTEIN IN EXPERIMENTAL LIVER REGENERATION: A NITROGEN BALANCE STUDY[1]

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Received for publication October 2, 1947

Experiments have been reported elsewhere upon regeneration of the liver after the performance of 69.4% hepatectomy on the partially protein-depleted rat (1, 2). The data indicated that the regenerative process could be subdivided into two phases. A certain portion of the lost liver substance was found to regenerate on a nonprotein diet during a 14-day postoperative period. In order to increase the degree of regeneration over and above that observed under conditions of protein starvation, the inclusion of an adequate protein in the postoperative diet was necessary.

The postoperative feeding of dietary protein led to a further increment of new liver substance. When the diet contained sufficient protein to promote a postoperative gain in body weight, considerable increments of new liver protein were observed. However, one diet which contained insufficient casein to promote any gain in body weight was associated nevertheless with a significant increment of new liver protein over and above that observed on a nonprotein diet. This group attracted attention owing to the possibility that an instance had been found where the liver was being favored preferentially by the small amount of protein in the diet.

A further finding that deficient food proteins, such as zein and gelatin, not only failed to support body weight gain but failed to promote liver protein recovery suggested that the intake of protein nitrogen could not be the determining factor in the promotion of liver-protein formation. Was the regeneration of new liver protein, as influenced by dietary protein, related to the utilization of ingested nitrogen by the body as a whole? Was there any constant allocation of dietary nitrogen to the regenerating liver? The nitrogen balance studies which follow represent an attempt to define the rôle of dietary protein in regeneration of the liver by relating the appearance of new liver protein to the nitrogen economy of the animal as a whole.

EXPERIMENTAL. Groups of 6 male Wistar rats averaging about 250 grams in body weight were fed a nonprotein diet for 14 days before the partial hepatectomy. After operation the same dietary mixture was modified to include a

[1] This work was made possible in part by the Merck Fund for Surgical Research.

This material was presented in part before the American Society of Biological Chemists in Chicago, May 18, 1947. Fed. Proc., 6: 257, 1947.

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food protein by direct substitution for sucrose. A standard postoperative interval of 14 days was used. All feeding was ad libitum except for two groups in which caloric restriction was required. In these two instances the daily allowance of food was offered at one time each day.

The methods described in detail elsewhere (1) were strictly adhered to. From data previously reported it was assumed that 69.4% ± 1.34% of the liver was removed at operation. Analytical values obtained from the removed lobes

TABLE 1. *Relation of nitrogen metabolism to liver protein in a 14-day regeneration period*

DIET	EXP. NO.	NO. OF RATS	SACRIF. SERUM PROTEIN	TOTAL FOOD INTAKE[1]	BODY WEIGHT			NITROGEN METABOLISM[1]						LIVER INCREMENT[1]		$\frac{N - N'}{SN} \times 100$[5]
					Initial	Oper.	Sacr.	Intake	Output: Urine	Output: Fecal	Output: Total	Balance	SN[4]	Protein	Protein N	
			%		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	mgm.	
Non-protein	19	4	4.16	40	250	197	170	0.00	0.23	0.05	0.28	−0.28	0.00	0.17	27.0	0.0
Non-protein ½ calories	20	6	5.46	20	248	200	158	0.00	0.26	0.03	0.29	−0.29	0.00	0.17	26.7	0.0
8.5% gelatin[2]	26	5	4.50	37	253	202	184	0.50	0.60	0.06	0.66	−0.16	0.12	0.15	23.7	0.0
Equivalent 10% casein low calories	28	4	5.96	19	246	204	171	0.77	0.81	0.07	0.88	−0.11	0.17	0.22	35.8	5.2
5% casein	8A	6	4.96	49	259	213	206	0.34	0.35	0.06	0.41	−0.07	0.21	0.22	36.0	4.2
4.4% casein + 1% methionine[3]	8B	5	4.87	53	255	212	209	0.37	0.32	0.06	0.38	−0.02	0.26	0.24	38.4	4.4
10% casein	14A	6	5.82	56	265	224	241	0.77	0.45	0.08	0.53	+0.24	0.52	0.30	48.2	4.1
9.3% casein + 1% methionine[3]	14B	5	5.58	52	264	225	247	0.71	0.35	0.07	0.42	+0.29	0.57	0.33	52.5	4.5
11% egg protein[2]	27	5	5.38	64	255	202	239	0.89	0.33	0.10	0.43	+0.46	0.74	0.37	59.7	4.4
18% casein	13A	4	5.85	59	245	199	229	1.47	0.93	0.09	1.02	+0.45	0.73	0.37	59.4	4.4
17.4% casein + 1% methionine[3]	13B	5	6.04	58	245	199	231	1.44	0.85	0.09	0.94	+0.50	0.78	0.40	64.8	4.8

[1] Grams per 100 grams initial body weight.

[2] Isonitrogenous with the 10% casein diet.

[3] Isonitrogenous with the preceding casein diet.

[4] Body nitrogen spared or saved above losses on the non-protein diet (0.28 gram). See figure 3, scale A.

[5] N − N′ signifies new liver protein N laid down minus that amount appearing on the non-protein diet (27.0 mgm.). The difference represents the portion of the new liver-protein nitrogen which is attributable to the dietary protein. See figure 3, scale B. The ratio shows the percentage of body nitrogen spared or saved which is allocated to the liver.

were multiplied by the factor 1.441 to give the values for the total liver at the onset of the operation. The values for the 'remnant' left in situ at the close of the operation were taken to be 30.6% of those for the total liver. Subtraction of the values for the remnant at operation from values obtained at sacrifice gave the increment of new substance appearing during the regenerative period.

Complete data on the composition of the liver for the majority of groups reported here have been tabulated elsewhere (2). Only the increment of new liver protein formed is recorded in table 1. The expression 'liver protein' is

used for convenience. It is calculated from the difference between the total and extractive nitrogen multiplied by the factor 6.25. It is recognized that this fraction contains nitrogen of other than protein origin, specifically nucleic acid and phospholipid nitrogen.

The basal non-protein diet was the same as that reported previously (1, 2). The food proteins used and their air-dry nitrogen percentage content have also been described (2).

Food consumption was measured daily and the nitrogen intake calculated. Daily collections of the urines were made for 4 days before operation and throughout the postoperative period. The animals were housed in circular wire-mesh cages. Excreta were collected on acidified filter paper placed in glass dishes. The papers were collected each morning at the same hour. The filter paper was divided and washed. The washings were brought to a suitable volume and aliquots taken for total nitrogen determinations by the micro-Kjeldahl method. Stools were pooled by groups and then stored in dilute acid, homogenized in a Waring blender, and aliquots analyzed similarily.

The nitrogen balance figures shown in figures 1 and 2 for the first 10 days of the pre-operative period were obtained from a single group of controls. In all instances the small amount of non-protein nitrogen contained in the synthetic vitamin supplement was ignored in computing the nitrogen balance.

Results. Results are summarized in table 1. The different dietary groups are arranged from above downward in the order of increasing increments of new liver protein formed. All values represent averages for each group. Each column dealing with nitrogen values shows the summation for the 14-day postoperative period in terms of average initial body weight. The main function of the table is to show that there is no uniform relationship between the amount of protein nitrogen consumed and the increment of new liver protein. On the other hand the nitrogen balance does show a relationship with the formation of new liver protein.

Control values for the protein content of the intact liver under the influence of the nonprotein diet have been reported elsewhere (1). These values may be summarized as follows expressed as liver protein per 100 grams of initial body weight: initial, 0.70 gram; after 14 days on the non-protein diet, 0.40 gram; after 28 days on the same diet, 0.37 gram.

Figure 1, on the left, shows the average results when 14 days of protein depletion were followed by the partial hepatectomy, the nonprotein diet being continued postoperatively. Body weight and serum proteins continue to decline. The nitrogen balance is negative throughout the course, with a small increase in nitrogen loss immediately after the operation. The reduction of the liver protein by both protein starvation and operation is shown. In the terminal column the stippled area represents the increment of new liver protein formed.[3]

[3] The differences between the liver protein increments, shown in the terminal liver-protein columns of figures 1 and 2, are highly significant by the t-test of Fisher (3) between successive groups (fig. 1), t = <.01; 5% casein versus 10% casein, t = <.01; 10% casein versus 18% casein, t = <.02.

On the right of figure 1 the postoperative diet has been modified to include casein at a level of 5% of the diet. Body weight and serum proteins have achieved a plateau after operation. The nitrogen balance after operation is indicated by the upper line and is seen to approach equilibrium but not to reach positive balance. The lower line delimiting the heavy diagonal shading shows the negative nitrogen balance of the group on the nonprotein diet. This is included to emphasize that the small amount of casein ingested, though not sufficient to

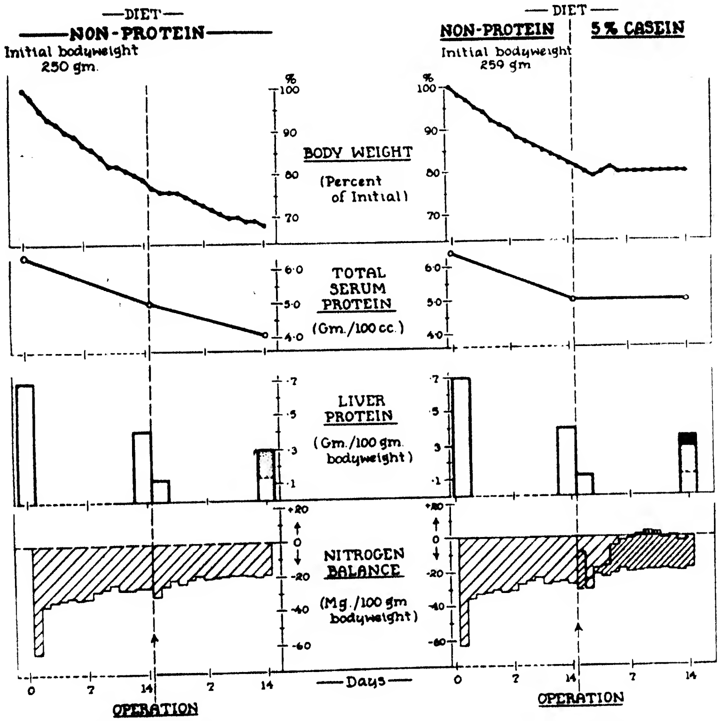

FIG. 1. AVERAGE VALUES FOR BODY WEIGHT, SERUM PROTEIN, LIVER PROTEIN AND NITROGEN BALANCE DURING A 28-DAY EXPERIMENTAL PERIOD. Broken ordinate lines indicate that associated values are derived from control groups. In the postoperative nitrogen balance of the group fed 5% casein, the heavy diagonal shading represents the nitrogen losses of the group on the non-protein diet.

promote a positive nitrogen balance, has spared considerable nitrogen which would otherwise have been lost. In the terminal liver protein column the stippled area is increment which would be expected to occur in the absence of dietary protein. The solid block on the column is the portion of the increment which is attributable to the ingestion of the casein. It must be conceded that the extra increment of liver protein indicated by the solid block cannot be a function of nitrogen made available above the requirements of maintenance, for in this instance maintenance requirements have not been surpassed.

Figure 2 on the left shows a group fed a 10% casein diet postoperatively. The pre-operative losses of body weight and serum proteins have been partially restored. A positive nitrogen balance has been established. The area of the positive nitrogen balance does not completely offset the pre-operative nitrogen loss. The increment of liver protein is larger. The surgical loss has been replaced, but the pre-operative dietetic loss has not. On the right of figure 2 casein has been fed at the 18% level. Body weight and serum proteins have risen further. The pre-operative body nitrogen loss has been replaced. A further rise of liver protein has occurred although regeneration to the pre-experimental level is not complete.

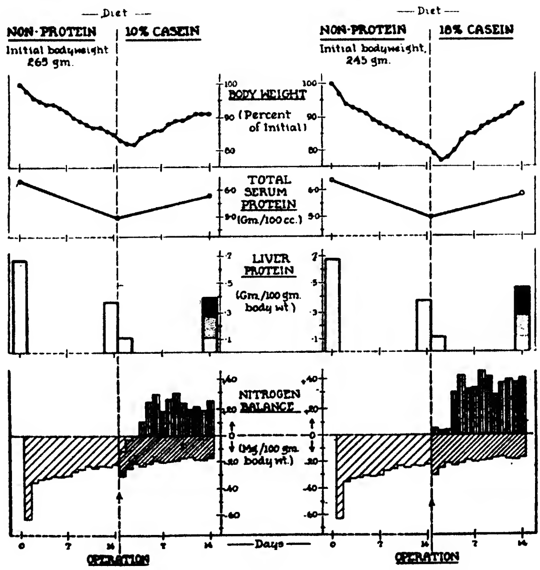

FIG. 2. SIMILAR VALUES TO THOSE OF FIGURE 1 FOR GROUPS FED 10 AND 18% CASEIN AFTER OPERATION. As in figure 1 the heavy diagonal shading in the nitrogen balance shows the nitrogen losses of the group on the non-protein diet postoperatively. Therefore, the sum of the heavy diagonal and vertical shading represents body nitrogen made available to the animal over losses on a non-protein diet.

As is seen in table 1, whole egg protein (experiment 27) at a dietary level isonitrogenous with 10% casein gave results which almost exactly superimpose upon those obtained with 18% casein. A further group (experiment 28) was fed casein at an intake identical with the 10% casein group (experiment 14A), but with carbohydrate so restricted that the caloric consumption was approximately 34% of the ad libitum intake. This group failed to sustain a positive nitrogen balance. Liver protein and nitrogen balance results were comparable to the 5% casein diet group.

The second group in table 1 (experiment 20) was restricted to 50% of the amount of the nonprotein diet which was eaten ad libitum. Although this

group lost more body weight as a result of the caloric restriction, there was no great increase in nitrogen excretion, and the replacement of new liver protein was not adversely affected.

The supplementation of the 5, 10 and 18% casein diets with extra methionine resulted in a consistent trend toward a more favorable nitrogen balance and a larger liver-protein increment. None of the 3 pairs of experiments, which were conducted concurrently, showed a statistically significant difference. Nevertheless, the consistent difference in the average results suggested that the methionine exerted a certain measurable effect.

Analysis of the data shown in table 1 disclosed a close correlation between the amount of new liver protein formed and the intake of casein nitrogen, provided ad libitum feeding was allowed. However, when an adequate intake of casein nitrogen was combined with restriction of calories, as in experiment 28, the amount of new liver protein formed was reduced. Again, the use of proteins other than casein led to the appearance of new liver protein in amounts which bore no close relation to the amount of protein nitrogen ingested. For example, the nitrogen consumption in the gelatin-fed group (experiment 26) was greater than in the 5% casein group (experiment 8A), yet the liver-protein increment was much smaller in the gelatin group. Conversely the egg-protein group (experiment 27) consumed less nitrogen than the group on 18% casein (experiment 13A), yet the increment of new liver protein was the same. It was apparent that the liver-protein increment was not related to the protein-nitrogen intake under all conditions studied. The most satisfactory correlation which the data could provide was found to be between the liver-protein increment and the nitrogen balance as shown in figure 3.

Figure 3 is a plot of the results of all individual animals which provided the average figures recorded in table 1. These average values are also plotted. The correlation is seen between the nitrogen balance and the increment of new liver protein. It will be noted that the scatter of results within each dietary group does not disturb the correlation. Individual variations in the summated 14-day nitrogen balance tend to be associated with a proportional difference in the liver-protein increment. Thus the variations in individual response, of which food intake would be an example, support rather than detract from the thesis that the amount of new liver protein formed is a function of the prevailing nitrogen balance.

The line formed by the plotting appears linear between the lowest groups on the nonprotein diet and the region of the group fed the 18% casein diet. Beyond this point there is a suggestion of an upward curvature. This may mean that the nature of the relationship becomes modified as the protein nutrition of the animal approaches the pre-experimental level. It has been noted in discussing figure 2 that the group fed the 18% casein diet had regained the body nitrogen which was lost in the pre-operative depletion period. At this stage of realimentation one might expect the nitrogen balance to become a decreasing variable, in which case the liver protein must achieve the final increase to the

pre-experimental level without a proportional further increase in the positivity of the nitrogen balance.

On the inner scales (*A* and *B*) of figure 3 the same values are shown as on the outer, the only difference being that the values for the ad libitum nonprotein diet group are taken as zero. These scales enable one to read off the constant proportionality expressed by the linear portion of the slope. Scale *A* now represents the nitrogen spared or saved for the body over losses occurring on a nonprotein diet. Scale *B* shows the liver protein nitrogen laid down in the liver over and above the amount which would be formed in the absence of dietary protein. It is seen that for every 100 mgm. of nitrogen spared or saved for the body, approximately 4.0 mgm. appear in the liver.

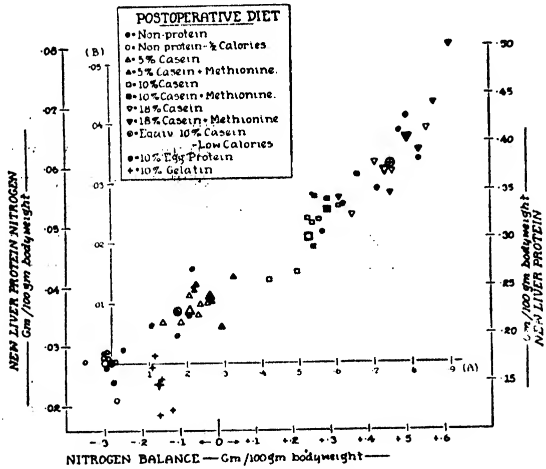

FIG. 3. CORRELATION BETWEEN THE SUMMATED 14-DAY POSTOPERATIVE NITROGEN BALANCE AND THE INCREMENT OF NEW LIVER-PROTEIN NITROGEN. Large points represent average values for each dietary group. Scales *A* and *B* show the same units as on the outer scales, but modified in that the average values of the group on the non-protein diet are taken as zero.

This ratio for the various groups is also shown in table 1, calculated as the percentage of nitrogen, made available to the body by virtue of the protein in the diet which appears in the liver. The relative constancy of the ratio leads to the conclusion that under the conditions of these experiments there is a constant allocation of the dietary nitrogen to the regenerating liver.

The concept is thus presented that dietary protein plays a rôle in liver regeneration by virtue of its function in sparing body nitrogen which would be lost on a protein-free dietary regimen. Furthermore, dietary protein may exercise this function irrespective of whether or not a positive nitrogen balance results. Finally, the most effective exercise of this function of protein demands conditions favorable to utilization of the protein nitrogen by the body as a whole. Conditions which have been specifically tested include an adequate associated caloric

intake and a protein capable of being adequately utilized by virtue of its amino acid composition.

An experiment which failed to conform to the correlation is represented by the group on the gelatin diet. From figure 3 it will be seen that the summated nitrogen balance is negative, though not so strongly negative as on the nonprotein diet. Despite the fact that some body nitrogen appears to have been spared over losses on the nonprotein diet, none of the spared nitrogen has been added to the liver. The liver-protein increment is actually a little less than appeared on the non-protein diet. Although the behavior of this group does not affect the general thesis, it does suggest that in this instance of an incomplete dietary protein, other factors may be operating which elude detection by the nitrogen balance technique.

SUMMARY

1. Rats were exposed to a 14-day period on a protein-free diet, followed by 69.4% hepatectomy. Various protein-containing diets were offered postoperatively over a 14-day period, during which nitrogen balances were determined. The increment of new liver protein formed postoperatively was determined.

2. The introduction of protein into the postoperative diet led to increases in new liver-protein formation in proportion to the effect of the diet upon the nitrogen balance of the animal as a whole. The relation of new liver protein formed to the nitrogen balance was linear on both sides on nitrogen equilibrium but became curvilinear as complete regeneration to pre-experimental levels was approached. In the linear portion of the slope the ratio of new liver-protein nitrogen over and above the level achieved on a nonprotein diet to the body nitrogen made available over losses on a nonprotein diet was approximately 4%.

3. Diets containing casein at 3 dietary levels, alone and with added methionine, casein with limited calories and whole egg protein gave results which conformed to the correlation described. An exception was found in a group fed gelatin in which a depression of liver protein regeneration occurred which was out of proportion to the effect upon body nitrogen metabolism as measured by the nitrogen balance.

4. Dietary protein exerts its effect upon the regeneration of liver protein as a function of the body nitrogen spared over nitrogen losses on a protein-free diet.

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EFFECT OF DIETARY PROTEIN UPON THE REGENERATION OF LIVER PROTEIN IN THE RAT[1]

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Received for publication October 2, 1947

Studies on the composition of the liver during a 14-day regeneration period after partial hepatectomy have been reported elsewhere (1). Casein-fed animals were compared with animals subjected to both pre-operative and postoperative protein starvation. It was found that a greater degree of regeneration took place in the protein-fed series but that considerable hepatic regeneration could occur in the absence of protein in the diet.

The literature does not afford quantitative data on the effect of dietary protein upon the composition of the various liver components during regeneration following partial hepatectomy. Previous authors have been concerned primarily with measurements of total liver mass (2), of cellular mitotic activity (3) or of the fat (4) or glycogen (5) component. No one group of previous experiments provides data on the composition of the gain on varied dietaries. Even the reappearance of liver protein has not been studied after partial hepatectomy.

In intact animals the regeneration of liver protein after a period of fasting of from 2 to 7 days has been measured by several investigators (6) who showed that high casein diets after fasting replaced the liver protein lost within 2 to 7 days. Harrison and Long (7) quantitated the regeneration of liver protein during a 4-day refeeding period after a 2-day fast. They showed that incomplete food proteins were less effective than casein or lactalbumin and provided evidence which suggested that complete regeneration of liver protein was contingent upon complete realimentation of the animal as a whole.

The losses in liver protein following starvation or protein depletion are well known. Addis, Poo and Lew (6) found a loss of 40% after 7 days of fasting in the rat. Kosterlitz and Campbell (8) found a similar loss on a nonprotein diet during the second week. In either type of depletion further prolongation of the starvation period was not associated with any further sharp decline in liver protein. Kosterlitz (9) showed that starvation losses of liver protein come largely from the cytoplasm with no decrease in the number of cells.

In the following experiments we have combined the nonprotein diet depletion technique with partial removal of the liver. In a previous report (1) we have

[1] This work was made possible in part by the Merck Fund for Surgical Research.

This material was presented in part before the American Society of Biological Chemists in Chicago, May 18, 1947. Fed. Proc., **6**: 299, 1947.

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shown that a 14-day period on a nonprotein diet reduced the liver protein of our rats by approximately 42%. Surgical removal of 69.4% of the remaining liver substance gave a net reduction of liver protein to approximately 17% of the pre-experimental value. Immediately after operation the food protein to be tested was introduced into the diet. The changes in amount and composition of the regenerated liver at the conclusion of a 14-day postoperative period were observed.

EXPERIMENTAL. Groups of 6 male Wistar rats averaging about 250 grams in body weight were fed (ad libitum) a nonprotein diet for 14 days before the partial hepatectomy. After operation the same dietary mixture was modified to include the test food protein by direct substitution for sucrose. A standard postoperative interval of 14 days was used, during which the protein-containing diet was fed ad libitum.

Full details of the methods employed have been described elsewhere (1). From data previously reported it was assumed that 69.4% ± 1.34% of the liver was removed at operation. Analytical values obtained from the removed lobes were multiplied by the factor 1.441 to give the values for the total liver at the onset of the operation. The values for the remaining 'remnant' left in situ were taken to be 30.6% of those for the total liver just prior to operation. Subtraction of values for the remnant at operation from values obtained at sacrifice gave the increment of new substance appearing during the regeneration period.

Liver analyses were made subsequent to operation and sacrifice for water, glycogen, lipid, total nitrogen and extractive nitrogen. The expression 'liver protein' is used for convenience. It is calculated from the difference between the total and extractive nitrogen multiplied by the factor 6.25. It is recognized that this fraction contains nitrogen of other than protein origin, specifically nucleic acid and phospholipid nitrogen.

The standard nonprotein diet was the same as that reported previously.[3] The air-dry nitrogen percentage of the various food proteins was as follows: casein, 13.80; gelatin, 16.22; zein, 14.80; gluten, 13.80; liver protein, 13.65; fibrin, 14.80 and whole egg protein, 12.48.[4]

Room temperature was maintained at approximately 28°C.

[3] Basal nonprotein diet, parts per 100 grams: sucrose, 91; codliver oil, 3; celluflour, 2; salts, 4. Vitamin supplement, mgm. per 10.0 grams of food: thiamin, riboflavin and pyridoxine, 0.1; nicotinic acid, 1.0; inositol, 6.0; p-aminobenzoic acid, 2.0; calcium pantothenate, 0.6; choline chloride, 20.0. We wish to thank Doctor Hans Molitor for a gift of the synthetic vitamins used.

[4] *Casein:* #453 fat-free casein, Casein Manufacturing Co., Bainbridge, N. Y.
Gelatin: special gelatin B-121-1, The Knox Gelatin Protein Products, Inc., Camden, N. J.
Zein: X grade, Corn Products Refining Co., New York, N. Y.
Gluten: wheat gluten, The Pure Gluten Food Co., New York, N. Y.
Liver Protein: liver protein digest #7-6075, Lederle Laboratories, Pearl River, N. Y.
Fibrin: bovine blood fibrin powder, The Wilson Laboratories, Chicago, Ill.
Egg Protein: defatted whole egg protein, obtained from Doctor James B. Allison and Doctor P. Swanson.

TABLE 1. *Effect of dietary protein upon regeneration of the liver in a 14-day postoperative period* (Grams per 100 grams initial body weight)

DIET	EXP. NO.	NO. OF RATS	BODY WEIGHT		TOTAL POSTOP. FOOD INTAKE[3]	TOTAL LIVER MASS[3]		COMPOSITION[1]								NEW LIVER PROTEIN FORMED[3]
			Initial	Post-oper. loss or gain		Oper.	Sacr.	Water Oper.	Water Sacr.	Glycogen Oper.	Glycogen Sacr.	Lipid Oper.	Lipid Sacr.	Protein Oper.	Protein Sacr.	
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Non-protein	7	5	254 ±11	−26	41 ±3	2.75 ±.17	2.17 ±.11	1.94 ±.14	1.54 ±.07	0.18 ±.05	0.14 ±.02	0.14 ±.02	0.14 ±.02	0.41 ±.01	0.29 ±.02	0.17 ±.01
Non-protein	19	4	250 ±5	−27	40 ±3	2.88 ±.39	2.34 ±.30	1.97 ±.27	1.63 ±.17	0.18	0.07	0.15	0.16	0.42 ±.03	0.30 ±.02	0.17 ±.01
[1]8.5% gelatin	26	5	253 ±10	−18	37 ±5	2.91 ±.17	2.04 ±.23	2.02 ±.10	1.44 ±.15	0.28	0.19	0.12	0.08	0.39 ±.02	0.27 ±.03	0.15 ±.03
[1]9.3% zein	16	6	252 ±12	−21	45 ±4	2.97 ±.29	2.09 ±.28	2.14 ±.21	1.48 ±.19	0.26	0.14	0.09	0.09	0.42 ±.04	0.30 ±.02	0.17 ±.01
5% casein	8A	6	259 ±10	−7	49 ±5	2.57 ±.13	2.44 ±.08	1.82 ±.08	1.73 ±.05	—	0.15	0.12	0.12	0.40 ±.01	0.35 ±.01	0.22 ±.01
[2]4.4% cas. + 1% meth.	8B	5	255 ±10	−3	53 ±5	2.51 ±.14	2.63 ±.10	1.78 ±.07	1.80 ±.06	—	0.18	0.12	0.20	0.40 ±.02	0.36 ±.02	0.24 ±.02
[1]10% gluten	18	5	246 ±16	+4	59 ±13	2.75 ±.32	2.69 ±.28	1.96 ±.21	1.88 ±.21	0.19	0.18	0.09	0.11	0.42 ±.03	0.39 ±.04	0.26 .04
[1]10.1% liver protein	17	5	247 ±7	+26	69 ±8	2.83 ±.20	3.02 ±.33	2.02 ±.16	2.11 ±.24	0.21	0.25	0.09	0.10	0.42 ±.03	0.43 ±.06	0.30 ±.05
10% casein	12	4	247 ±9	+18	55 ±3	2.63 ±.21	2.79 ±.31	1.85 ±.14	1.95 ±.17	0.19	0.22	0.17	0.11	0.37 ±.02	0.42 ±.02	0.30 ±.02
10% casein	14A	6	265 ±14	+17	56 ±7	2.60 ±.23	2.78 ±.34	1.84 ±.17	1.93 ±.22	0.18	0.23	0.10 ±.01	0.10 ±.03	0.38 ±.03	0.42 ±.03	0.30 ±.03
[2]9.3% cas. + 1% meth.	14B	5	264 ±20	+22	52 ±4	2.69 ±.25	3.12 ±.23	1.90 ±.19	2.15 ±.15	0.18	0.29	0.12 ±.01	0.11 ±.03	0.39 ±.02	0.45 ±.03	0.33 ±.03
[1]9.3% fibrin	15	6	251 ±11	+34	69 ±4	2.96 ±.18	3.03 ±.18	2.10 ±.13	2.11 ±.12	0.26	0.21	0.08	0.09	0.42 ±.01	0.50 ±.03	0.37 ±.02
[1]11% egg protein	27	5	255 ±9	+37	64 ±8	3.04 ±.57	3.05 ±.28	2.14 ±.35	2.16 ±.18	0.23	0.18	0.14	0.11	0.41 ±.02	0.50 ±.03	0.37 ±.04
18% casein	13A	4	245 ±18	+30	59 ±6	2.36 ±.19	2.78 ±.17	1.66 ±.19	1.92 ±.16	0.15	0.16	0.13	0.08 ±.01	0.39 ±.03	0.49 ±.04	0.37 ±.04
[2]17.4% cas. + 1% meth.	13B	5	245 ±9	+32	58 ±5	2.38 ±.35	2.95 ±.41	1.71 ±.19	2.07 ±.36	0.12	0.15	0.11	0.10 ±.03	0.39 ±.03	0.52 ±.07	0.40 ±.07
27% casein	11	2	260 ±14	+46	61 ±2	2.53 ±.13	2.99 ±.20	1.81 ±.08	2.07 ±.17	0.15	0.17	0.14	0.10	0.39 ±.01	0.56 ±.03	0.44 ±.03
Operation average		78	253 ±12			2.71 ±.24		1.92 ±.17		0.19		0.12		0.40 ±.02		

[1] Isonitrogenous with the 10% casein diet.
[2] Isonitrogenous with the preceding casein diet.
[3] All values shown are averages for each experimental group. Where individual values were determined, the standard deviation is shown. S.D. = $\sqrt{\frac{\Sigma \Delta x^2}{n-1}}$

RESULTS. Ninety-five rats were used.[5] The results from 78 animals which completed the experimental period are shown in table 1. The average values for the amount and composition of the liver at the time of operation agree closely with those already reported for animals similarily prepared. The averages of various dietary groups are recorded from above downward in the order of increasing increments of new protein appearing in the liver. It will be observed that the values for the total liver mass at sacrifice show a similar trend, but that maximal values have been reached with the 10% protein diets. The same applies in general to the values for water. The glycogen values at sacrifice are greatest in the rats receiving 10% protein diets and tend to fall with increasing amounts of protein in the diet. The lipid values, as would be expected, are greatest in the rats on the nonprotein diet. There is very little variation at sacrifice in the absolute amount of hepatic lipid present in the rats on the protein-containing diets.

The increments for the water, glycogen and lipid components may be calculated readily. When this is done it is apparent that the glycogen increment is greatest in the intermediate range of dietary protein consumption and somewhat less at low and high levels of intake. The lipid increment is quite constant in all groups. The principal components contributing to the gain, as food protein consumption increases, are protein and water.

The amount of new liver protein appearing on the nonprotein diet following partial hepatectomy was 0.17 gram. Casein fed at increasing levels resulted in increasing increments up to 0.44 gram on the 27% diet. The introduction of additional methionine to the casein diets led to small but consistent increases in the new protein. The other proteins were fed at levels isonitrogenous with the 10% casein diet. The results in terms of liver-protein increment appear to parallel the known ability of these proteins to realiment the protein-depleted rat, an ability known to be related to their amino acid composition (10, 11). Thus gelatin and zein resulted in no increase above that observed on the nonprotein diet, while fibrin and whole-egg protein show a greater increment than casein fed at a similar level.

The liver protein removed by operation has been replaced by the use of the 10% liver protein and 10% casein diets. The more effective diets have surpassed this mark, not only replacing the loss incurred at operation but also a portion of the pre-operative dietetic loss. Control data already reported (1) have shown that intact animals prior to protein depletion have a liver protein of 0.70 gram per 100 grams of body weight. This level was not regained in any of the feeding experiments following partial hepatectomy.

The average total food intake during the 14-day preoperative period of nonprotein feeding was 55.0 grams ± 6.0 grams per 100 grams of initial body weight. The animals generally ate the nonprotein diet in large amounts for 3 to 5 days,

[5] Two animals died at operation and 12 in the postoperative period. Three others were removed from the series, one because of jaundice and anorexia, two because of accidents during the nitrogen analyses.

after which the intake gradually declined. The average preoperative weight loss amounted to 18% ± 2% of the initial body weight. Variations in total postoperative food intake are apparent from table 1. The two groups on the nonprotein diet ate 41.0 and 40.0 grams per 100 grams of initial body weight. The addition of protein to the mixture resulted in increasingly large food consumption, up to 69.0 grams in the 10% liver protein and fibrin groups.

Postoperative body weight changes are also shown in table 1. Animals on the nonprotein diet continued to lose weight, as did those on gelatin and zein diets. The ingestion of the 5% casein diet greatly reduced the weight loss. Although no gain occurred in body weight in these groups, a substantial increase in liver protein was observed. At higher levels of protein intake the increase in both body weight and liver protein appeared roughly parallel.

Attention is drawn to the groups fed 5% casein with and without methionine. Without showing a gain in body weight they displayed a significant increase in liver protein over the groups on the nonprotein diet. These data suggest a selective action of the dietary protein upon the liver which was not readily reconciled with the apparent parallelism between body weight and liver-protein recovery at the higher levels of protein intake. Further studies have been undertaken, using the nitrogen balance as an index of dietary protein utilization, in an attempt to define more precisely the relationship between liver protein regeneration and the protein metabolism of the animal as a whole. These results will be reported later.

SUMMARY

1. Rats subjected to a period of 14 days on a nonprotein diet, followed by 69.4% hepatectomy, were found to regenerate a constant amount of new liver substance while consuming a nonprotein diet during a postoperative period of 14 days. All normal liver components, including liver protein, were found to contribute to the regeneration without gross distortion of their normal proportions.

2. The inclusion of the more adequate food proteins in the postoperative diet was associated with significant increases in amount of regeneration over and above that which occurred on a nonprotein diet. Such increases occurred predominantly in the water and protein components.

3. Progressive increases in the intake of a single protein (casein) were associated with progressive increases in liver protein. The introduction of extra methionine at a 1% level into each casein diet appeared to augment the effect of casein alone to a small but consistent degree.

4. The feeding of other proteins at dietary levels isonitrogenous with the 10% casein diet led to widely varied effects. Gelatin and zein led to no more regeneration of liver protein than occurred on a nonprotein diet. Wheat gluten showed a moderate increase. Liver protein was comparable to casein. Fibrin and whole-egg protein led to a greater amount of new liver protein than did casein at the same dietary level.

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WORKING CAPACITY IN PATIENTS WITH ORTHOPEDIC HANDICAPS FROM POLIOMYELITIS

I. Energy Expenditure in Walking at Various Speeds and Grades[1]

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In spite of the progress in physical therapy, the proportion of patients with remaining orthopedic handicaps resulting from infantile paralysis is large enough to be of concern for their employment as well as their capability to meet the demands of everyday life. During and after the war, much work has been done on rehabilitation of patients with mutilations from war or industrial accidents (1), from which undoubtedly patients with orthopedic handicaps from other causes will benefit. However, the approach has been entirely empirical so far. It seems that an assessment of the working capacity of such patients, based on the analysis of fundamental physiological functions, would be a superior procedure. It is the purpose of this communication to provide some experimental data for this approach. Working capacity is a complex integral of various functions (2, 3), but their importance for patients with orthopedic handicaps differs widely. For instance, there is no reason to assume a decrease of pulmonary or cardiovascular capacity. Thus, the task of determining the physiological basis of working capacity is primarily restricted, in fact to two areas—energetics and motor coordination. Both are intimately interrelated.

In the present work both energetics and motor coordination were studied in walking on a motor-driven treadmill at various speeds and grades. Walking is, perhaps, the most important type of physical activity in its general significance. Motor coordination was investigated by means of high-speed motion pictures; the results obtained will be published elsewhere. The present communication is concerned with the energetics of walking.

To our knowledge, only Steindler (4) has investigated the energy cost of walking on a treadmill in patients with handicaps from infantile paralysis. The increase of the energy expenditure in 13 patients (from 6 to 20 years of age) was stated to range from 16.8 to 150.8% above 'normal.' The speed of the treadmill was not given, and obviously only one speed was investigated at zero grade (horizontal). No comparative data of normal subjects, investigated in the same laboratory, were communicated; the comparison refers probably to some 'average' data in the literature.

Obviously, crippled persons will show a wide range of energy expenditure in walking, depending on the degree of orthopedic handicap.

[1] This work was aided by a grant from the National Foundation for Infantile Paralysis.

Patients and subjects. In our opinion, a thorough investigation of the trends of the energy expenditure with variation of speed and grade will be much more revealing as to the physiological consequences of the handicap than investigation of a larger number of patients only at one speed and grade could be. Our investigations were performed on two patients who were at the extreme limits of orthopedic handicap. Anthropometric data on these patients are given in table 1.

One patient, J. D. (21 years of age), had recovered from poliomyelitis 10 years ago. Except for an atrophic right leg, J. D. was of an athletic type of body build with well-developed muscles. All muscle groups of the right leg were markedly atrophic and the length of the leg was 11 cm. shorter than the left leg. The patient used braces, although he was able to walk without them for short distances. His impairment was such that the right leg appeared to be passive in walking, acting like a pivot, around which the left leg was swung. It was only with some apprehension that a first test was made at a slow speed; we had the impression that his degree of impairment was about the limit for an investigation of this type.

TABLE 1. *Height, weight and measurements of circumferences (cm.) on subjects J. D. and E. B.*

	J. D.	E. B.
Left leg, middle thigh	63	41
Right leg, middle thigh	44	44.5
Left leg, middle calf	42	34
Right leg, middle calf	21.5	34
Height cm	182	168
Weight kgm	77.4	56.5
Chest	100	83
Abdomen	85	67.5

The other patient, E. B. (17 years of age), had had a remarkable recovery from a paralysis of both legs during the acute phase one year before our experiment. His gait appeared to be perfectly normal at all speeds and grades, even at 4 m.p.h. and 10% grade, which is strenuous even for a normal subject. E. B. was of the asthenic type.

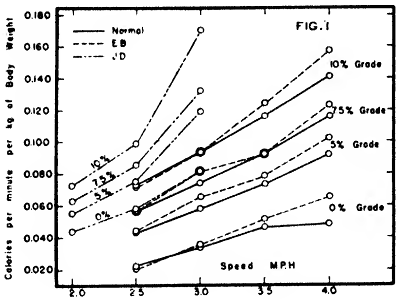
It seemed that by investigation of two patients at the extremes of recovery a reasonable exploration of the problem as a whole could be made and that investigation of cases with intermediate degrees of handicap would add comparatively little essential information. Investigation of patients with intermediate degrees of handicap, however, is desirable for the analysis of working capacity of the *individual* patient.

For comparison, the values of two normal subjects at 16 variations of speed and grade were available (5), ranging from 2.5 to 4 m.p.h. and 0 to 10% grade. These two men were very similar to patient J. D. in age, height and weight. The values obtained on groups of 7 and 47 subjects at two variations (2.5 m.p.h., 0 grade and 3.5 m.p.h. 10% grade) coincided well with the data of these two subjects. Patient J. D. was not able to walk at speeds higher than 3 m.p.h. For

this reason, he was investigated at 12 variations of speed and grade ranging from 2 to 3 m.p.h. and from 0 to 10% grade. No normal values at 2 m.p.h. are available; this speed is very slow for a normal subject, but was J.D.'s usual speed of walking.

METHOD

The method was the same as that used in our study in normal subjects (5), except that the walking period was 15 to 20 minutes instead of 30 minutes. The sample collection was started after 7 or 8 minutes walking, so that a steady state could be assumed. The existence of a steady state was controlled on several occasions by means of a second measurement of energy exchange, made a few minutes after the first one, while the subject continued walking. The first actual experiment was performed after two or three trial experiments, in order to eliminate technical training. The variability between repeat experiments of the



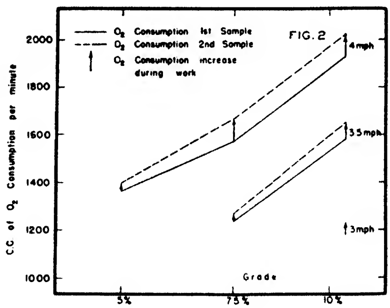


FIG. 1. Increase of energy expenditure of two poliomyelitis patients (E. B. and J. D.) at different grades and speeds in walking on a treadmill, compared to the average of two normal subjects.

FIG. 2. Breakdown of the steady state of oxygen consumption of poliomyelitis patient E. B. in walking at higher speeds and grades. The arrows show the increase of oxygen consumption from a first sample (*solid line*), taken after 8 minutes walking, to a second sample, taken after 14 to 15 minutes walking (*broken line*).

same variation on different days was within normal limits (± 3% of the mean). No training trend was apparent.

RESULTS

Energy cost. The excess energy expenditure (above the basal rate) was calculated in terms of Cal. per meter of distance walked per kgm. body weight. The calculation per kgm. body weight was necessary because of the large difference of the body weight between E. B. and the other subjects. It was shown (5) that this procedure of calculation decreases the interindividual variability considerably. Figure 1 shows the values at four grades (0 = horizontal, 5%, 7.5% and 10%) plotted with increasing speed. The normal curves (N) were obtained as averages of the two normal subjects, which was permissible, since the values of A.B. and D.M. coincided very closely at all 16 variations. It can be seen that the values of E. B. are practically identical with the normal values (N) up to a

speed of 3.5 m.p.h. In view of the great weight difference between E. B. and the two normal subjects the agreement is amazing. At 4.0 m.p.h., however, the curves of E. B. slope up somewhat steeper indicating that at this speed E. B. approaches his limit more closely than normal subjects do.

The energy expenditure of J. D. was markedly higher at all variations of speed and grade investigated. At the slow speed of 2.0 m.p.h., 0 grade, he used as much energy as a normal subject at 3.5 m.p.h.; his energy expenditure at 3.0 m.p.h. and zero grade is so high that it is improbable, from the trend of the curves, that a normal subject would approach it even at a considerably higher speed. Perhaps even more significant is the steep increase of the energy expenditure in grade walking at 3 m.p.h., especially at 10% grade. The large waste of energy is due to the extensive use of auxiliary muscle groups, working under unfavorable conditions of leverage, and performing extra movements for maintenance of equilibrium, which are only indirectly used for the movement of the body as a whole.

TABLE 2. *Relative energy expenditure of two poliomyelitis patients as percentages of average normal values, during walking on the treadmill*

SUBJECT	GRADE	SPEED (m.p.h.)			
		2.5	3.0	3.5	4.0
E. B.	0	91	103	111	135
	5	102	112	107	111
	7.5	98	109	100	106
	10	99	100	107	111
J. D.	0	263	237	—	—
	5	174	205	—	—
	7.5	149	178	—	—
	10	136	182	—	—

This means that a patient with such a degree of orthopedic handicap does rather heavy work when walking at a moderate speed. At 3 m.p.h., 10% grade, J. D.'s energy expenditure exceeds that of normal subjects at 4 m.p.h., 10% grade, a variation which many normal subjects would not be able to maintain for a prolonged time. In terms of the ability to expend energy J. D. belongs in the athletic class.

We expected that the percentage difference of the energy expenditure between normals and poliomyelitis patients would increase with the grade, but this was not the case (table 2). In E. B. the increase of the energy expenditure at 4 m.p.h. was most pronounced at zero grade. In J. D. the percentage increase was most pronounced at zero grade both at 2.5 and 3.0 m.p.h. and declined with increasing grade. The rate of decline is more pronounced at 2.5 m.p.h., probably because other factors counteract the relative saving of energy in grade walking at the higher speed of 3.0 m.p.h. This is indicated by the trend of the curves at 3 m.p.h. (fig. 1). The relative greater economy of grade walking than of horizontal walking suggests that a substantial proportion of the auxiliary movements in horizontal walking can be utilized for climbing. This should not be interpreted,

of course, that grade walking is easier than horizontal walking for the poliomyelitis patient, since the absolute energy expenditure increases with the grade.

Net efficiency. In our previous communication (5) we calculated the net 'efficiency of climbing' from the caloric equivalent of the amount of body lift divided by the difference of energy expenditure between grade and horizontal walking. This calculation, first suggested by Zuntz, was used by Benedict and Murschhauser (6), Smith (7) and others and may be regarded as a common expression for the efficiency of climbing.

Some objections were raised (5) against such method of calculation, which can now be further substantiated. The net climbing efficiency was calculated for J. D. at a speed of 2.5 m.p.h. for 5%, 7.5% and 10% grade and compared to the values of the normal subjects A. B. and D. M. (table 3). At all grades, the climbing efficiency of J. D. was much better than that of the normal subjects, but this was due to the large energy waste of J. D. at horizontal walking. A conclusion of a better climbing efficiency of J. D. would be inaccurate, since his energy expenditure is higher at all variations. We feel that these results discredit the common procedure of calculating net climbing efficiencies as well as differential efficiencies between two loads in other types of work (8, 9, 10).

TABLE 3. *Mechanical 'efficiency' of climbing for poliomyelitis patient J. D., compared to two normal subjects (A. B. and D. M.) at a speed of 2.5 m.p.h.*

SUBJECT	EFFICIENCY IN PER CENT		
	Grade		
	5%	7.5%	10%
A. B.	31.7	31.4	30.0
D. M.	28.3	31.6	28.7
J. D.	40.9	42.3	38.3

Effect of braces. The effect of braces on the energy cost was investigated with J. D. at the slowest speed (2 m.p.h.) and zero grade. Instead of an average of 3.4 excess Cal. per minute with braces, J. D. used 5.4 excess Cal. per minute. The pronounced increase of energy consumption when walking without braces explains J. D.'s inability to walk without braces for more than a short distance. In another experiment at 2 m.p.h. and zero grade, J. D. held on to a support mounted beside the treadmill. This type of support, roughly comparable to that which might be obtained by means of a solid walking stick, did not produce any saving of energy.

Maintenance of steady state. The normal subjects were able to maintain a steady state of oxygen consumption at all combinations of speeds and grades for at least 30 minutes. J. D. was able to maintain a steady state of oxygen consumption for at least 20 minutes up to 2.5 m.p.h. and 7.5% grade. At 2.5 m.p.h. and 10% grade, the maintenance of the steady state was not controlled by gas analysis, but there was no increase of the blood lactic acid (6.5 mgm. per 100 cc.) above the resting level (6.7 mgm.) after the end of the exercise. It is, therefore,

probable that J. D. was able to maintain a steady state also at this variation. Unfortunately, no determinations were made at 3 m.p.h. J. D. was, aside from the atrophy of his right leg, an athletic individual, and the results at 2.5 m.p.h. led us to believe that he was able to maintain a steady state at all speeds he was able to walk. This might well be the case, but when different results were obtained with E. B., patient J. D. was not available for further study.

Figure 2 shows the results obtained in E. B.; the solid lines represent the oxygen consumption at the time of the first sample with increasing grade, and the dotted lines the oxygen consumption at the time of the second sample, which was taken a few minutes after the first one. The vertical arrows between both lines represent the breakdown of the steady state. The difference between the first and the second sample increases with grade and speed. At all other variations E. B. was able to attain and maintain a steady state. Although the difference between first and second sample increases with the level of oxygen consumption, the level of oxygen consumption is not the only factor responsible for the failure to maintain a steady state. At the same oxygen consumption the breakdown of the steady state seems to be more pronounced at higher grades.

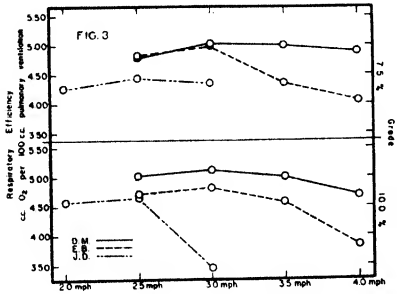
It is interesting that E. B.'s energy cost was normal at all these variations except 4 m.p.h., 10% grade, where it was slightly increased. The maintenance of the steady state is obviously a different and more sensitive criterion for muscular weakness than the energy cost. The subject would not have been able to continue walking at any of these variations for a much longer time; in fact, he completed the hardest variations only with effort.

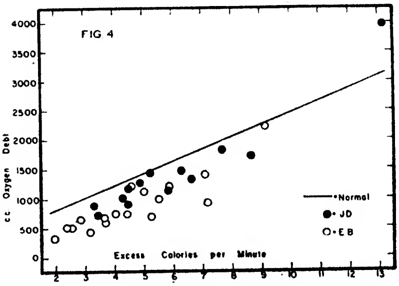
Respiratory efficiency. The respiratory efficiency, expressed as cc. oxygen consumption per 100 cc. pulmonary ventilation, ranged in the normal subjects A. B. and D. M. from 4.72 to 5.40 and from 4.67 to 5.12, respectively, in the various combinations of speed and grade investigated. In E. B. the respiratory efficiency ranged between 3.83 and 5.03, and in J. D., between 3.46 and 4.66. The values of E. B. overlap with the normal values but are lower on the average, while the values of J. D. are so low that there is no over-lapping between his values and the normal values. The values of A. B. and D. M. are by no means high for normal subjects. At 3.5 m.p.h., 10% grade, values of 32 normal subjects were available for comparison. The average of this group was 5.54 with a standard deviation of $\pm .412$. The values of A. B. and D. M. at this variation were 5.21 and 4.98, compared to 4.58 in E. B. and 3.46 in J. D. at 3 m.p.h. (no value at 3.5 m.p.h. was available for J. D.).

More interesting than the general range or the average values at a given variation of speed and grade is the trend of respiratory efficiency with speed. In A. B. there was a tendency of an increase of respiratory efficiency with the increase of energy expenditure (5). In D. M. there was no particular trend in horizontal walking, while in grade walking the respiratory efficiency had a tendency to increase slightly up to 3.0 m.p.h. and to drop with higher speeds. This trend was more apparent with increasing grade (fig. 3). In E. B. the drop with increasing speed was much more pronounced both at 7.5% and 10% grade. In J. D. there was only a slight drop at 7.5% grade and 3 m.p.h., but his respiratory

efficiency was much lower at all speeds, and it drops at 3.0 m.p.h. where the normal subjects as well as E. B. attain the maximum. At 10% grade, 3 m.p.h., J. D.'s respiratory efficiency drops with a much steeper slope than that of E. B. at 4.0 m.p.h.

Oxygen debt and work pulse rate. Figure 4 shows the oxygen debt of E. B. and J. D., plotted against excess calories per minute, compared to the normal slope (solid line) obtained from the values of A. B. and D. M. The normal slope could





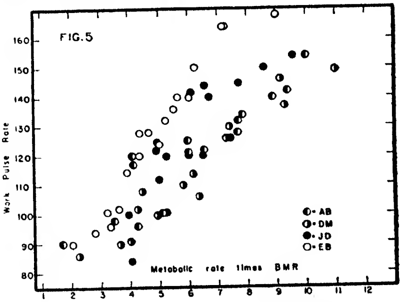


FIG. 3. Trends of respiratory efficiency (cc. O_2 per 100 cc. pulmonary ventilation, ordinate) with increasing speed (abscissa) at 7.5% grade (*upper part*) and 10% grade (*lower part*) of two poliomyelitis patients (E. B. and J. D.), compared to one normal subject (D. M.).

FIG. 4. Amount of the oxygen debt, cc. O_2 (ordinate), with energy expenditure (abscissa) of two poliomyelitis patients (E. B. and J. D.), compared to the average trend of two normal subjects.

FIG. 5. The pulse rate during work, beats per minute (ordinate), with the increase of the metabolic rate expressed as multiple of the basal metabolic rate (abscissa) of two poliomyelitis patients (E. B. and J. D.) and two normal subjects (A. B. and D. M.).

be expressed as the equation $y = 1.981 \times +42.803$ with a very high correlation, $r = 0.977$, between oxygen debt and energy expenditure. The values of E. B. and D. J. run approximately parallel to the normal slope but are below the normal average values, except one value of J. D. which is definitely higher. This value was obtained at the hardest combination (3 m.p.h. and 10% grade) J. D. was able to walk. The excessive oxygen debt, together with other data (trend of energy expenditure, respiratory efficiency), indicates clearly that this variation exceeded his capacity. The work pulse rate was plotted against the increase of

the metabolic rate for all four subjects (fig. 5). At all rates of energy expenditure above about 5 to 6 times the basal metabolic rate, the pulse rate of J. D. and especially of E. B. is above that of the normal subjects. The relative tachycardia in work of E. B. could be readily explained as simply that of a non-athletic individual; this explanation does not apply in the case of J. D.

Rate of steps. In normal subjects the rate of stepping increased with the speed, while the grade had no particular effect, i.e., the rate of steps was the same for all grades at a given speed (5). This was observed not only in E. B., but also in J. D. (table 2), which is surprising considering the degree of J. D.'s handicap. Table 4 shows the average rate of steps at different speeds. There is no difference between E. B. and the normal subject at all speeds; even J. D.'s values coincide at 2.5 and 3.0 m.p.h. At 2.0 m.p.h., his rate is higher than that of the normal subjects. It is surprising that this difference appears at the slowest speed.

TABLE 4. *Rate of steps at different speeds and grades, poliomyelitis patient J. D.*

GRADE	SPEED (m.p.h.)		
	2	2.5	3.0
0	84	92	106
5	86	92	106
7.5	84	92	108
10	84	92	108

DISCUSSION

The data obtained reveal various differences between normal men and patients with handicaps resulting from infantile paralysis. An attempt of a functional evaluation appears to be worth while, the more so as probably some generalization is possible for similar orthopedic handicaps resulting from other causes.

The level of the energy expenditure at a given variation characterizes the degree of handicap of a given patient, but this differentiation is most pronounced at horizontal walking. The level of energy expenditure at a given speed and grade fails to indicate fatigue or tolerance trends or the increasing effort involved in grade walking. The increase of the energy expenditure with speed is a much better criterion for the limit of tolerance. The trend of the curves would indicate that 4 m.p.h. is the limit of tolerance for E. B., and 2.5 m.p.h. for J. D., especially at 10% grade.

The level of energy expenditure appears to be a good index for the relief obtained by means of braces. It is possible that this method could be used to compare different types of braces. The ability to maintain a steady state of oxygen consumption appears to be a good index for fatigue trends. The comparatively low oxygen debts of both J. D. and E. B. indicate that the breakdown of the steady state (which was demonstrated only in E. B.) is not due to cardiovascular insufficiency. Considerable previous work (reviewed by Simonson and Enzer, 2) shows that an increased oxygen debt is one of the most typical and consistent findings in patients with cardiovascular insufficiency. The maximum

level at which the steady state can be maintained in normal subjects depends on the cardiovascular capacity for oxygen transport. Patients with orthopedic handicaps are obviously not able to reach this limit. Therefore, the increase of the oxygen consumption with continuation of walking is obviously due to the increasing use of auxiliary muscle groups. This might explain why in E. B. the breakdown of the steady state was not associated with an increased oxygen debt. The formation of the oxygen debt is due to the relative lag of blood circulation behind the peripheral oxygen requirement in the first minutes of work. At the time the auxiliary muscles are used the circulation has reached a sufficient rate to guarantee an adequate oxygen supply to the suxiliary muscles.

The drop of the respiratory efficiency appears to be a good criterion for the limit of working capacity and for fatigue of patients with orthopedic handicaps as well as in normal subjects. It also is decreased in patients with pulmonary or circulatory insufficiency (cf., 2). However, there is no reason to assume any cardiovascular insufficiency in E. B. and J. D., since the oxygen debt was not

TABLE 5. *Average rate of steps per minute at different speeds of two normal subjects (A. B. and D. M.) and two poliomyelitis patients (E. B. and J. D.), for all grades*

SUBJECT	SPEED m.p.h.				
	2.0	2.5	3.0	3.5	4.0
A. B.	76	96	111	122	130
D. M.	76	89	99	103	116
E. B.	—	95	108	119	125
J. D.	85	92	107	—	—

increased. On the contrary, the increased pulmonary ventilation might have kept the oxygen debt comparatively small; such an effect of pulmonary hyperventilation has been observed in normal subjects (11).

The excessive increase of pulmonary ventilation in E. B. and J. D. at moderate levels of work accomplishment is probably due to central or reflex hyperpnea; a similar effect occurs in normal subjects at a higher work level. The reflex mechanism for the regulation of pulmonary ventilation in exercise (12) might well be exaggerated in patients with orthopedic handicaps or muscular weakness. It is possible that a similar mechanism is involved in the relatively high work pulse rate of the poliomyelitis patients.

SUMMARY

1. The energy expenditures of two poliomyelitis patients, one clinically completely recovered from paralysis (E. B.) the other one with an almost atrophic right leg (J. D.), were compared to those of two normal subjects during walking on a treadmill at different speeds and grades.

2. E. B.'s energy expenditure, expressed as excess Cal. per meter distance per kgm. body weight, coincided with the normal values at all variations of speed and grade up to 3.5 m.p.h. with a slight tendency to higher values at 4.0 m.p.h.

3. J. D.'s energy expenditure when walking with braces exceeded the normal values (1.5 to 2.6 times) at all variations investigated (up to 3 m.p.h.), but the relative increase was higher in horizontal than in grade walking; a part of the energy waste in horizontal walking could be utilized for climbing.

4. Without braces, J. D.'s energy expenditure was considerably higher than it was with braces.

5. E. B. was not able to maintain a steady state at 3.5 m.p.h., 7.5% and 10% grade, and 4 m.p.h. at all grades. The normal subjects were able to maintain a steady state at least up to 30 minutes at these variations.

6. The respiratory efficiency (cc. oxygen consumption per 100 cc. pulmonary ventilation) of J. D. was definitely lower than that of the other subjects at all variations; a pronounced drop of the respiratory efficiency occurred in both J. D. and E. B. at the higher speeds and grades.

7. The oxygen debt of E. B. and J. D. followed a similar trend with the energy expenditure to that observed in normal subjects, but was comparatively low.

8. The work pulse rate tended to be high in both E. B. and J. D. at all levels of energy expenditure above 5 to 6 times the basal metabolic rate.

9. The rate of steps increased, as in normal subjects, with the speed but was independent of the grade.

10. An attempt is made to discuss the data with a view of evaluation of working capacity in persons with orthopedic handicaps.

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FORCES EXERTED AT DIFFERENT VELOCITIES IN HUMAN ARM MOVEMENTS

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Received for publication July 12, 1947

In a study of the dynamics of isolated frog muscle Hill (1) developed a hyperbolic relationship between the force exerted and the speed developed, expressed as $(P + a)(v + b) = \text{a constant} = (P_0 + a)\,b$, where P is force, v is the velocity of shortening, P_0, the isometric tension and a and b, constants. This equation was verified by mechanical and by heat production studies. Hill (2) also analyzed the previously obtained data for contractions of the human arm against an inertia wheel (3–5) and found that these data could be adequately expressed by the hyperbolic equation of frog's muscle. It was recognized, however, that the available data covered only a limited range of forces and velocities, and Hill suggested in the same paper (2) and by personal communication to us that the data be extended in order to provide a fairer test of the applicability of the equation to human muscle.

METHODS

The relationship of force to velocity in maximal voluntary flexions of the human forearm was studied. Three types of opposing load were used: *a*) the reaction of various moments of inertia; *b*) constant (isotonic) torques about the elbow joint; and *c*) isotonic forces applied parallel to the forearm flexor muscles.

APPARATUS

Isotonic lever. The apparatus shown in figure 1A was designed to supply constant force (in wire E) with as little inertia in the system as possible. The force could be used directly or converted to a constant torque to oppose the arm's movement. The first stage lever (L_2), connected directly to the piano wire (E), was made of aluminum alloy. This lever was designed by Mr. Thomas Rae of the Grumman Aircraft Corp. L. I., N. Y. The second stage lever (L_1) was of steel plate construction and was connected to the light lever by $\frac{1}{8}''$ airplane cable (D) and directly to the spring (S) below its fulcrum. The levers were mounted on steel shafts ($\frac{1}{2}''$ and $\frac{3}{4}''$ for L_2 and L_1, respectively). The shafts turned in bearings mounted in steel plates (P and P_1) which were bolted to the 4 x 6″ pine framework. Details of construction of the levers are given in figure 1C. Ball bearings were mounted in the levers for connection of wire E, the spring and cable D. A steel shaft through the bearing engaged a yoke spanning the lever, which, in turn, was attached to the wire or cable. Self-aligning ball bearings were used throughout. The spring was connected to the ratchet windlass (W) by flexible $\frac{1}{8}''$ airplane cable (C). A variety of tensions in wire E were produced by

winding up cable C to varying lengths. The spring (length 45″) when extended to the greatest recommended length (71″) exerted a force of 1500 lbs. Since the total mechanical advantage between spring and wire E was 30, the maximal tension that could be produced in E was approximately 50 lbs. (23 kgm.). For

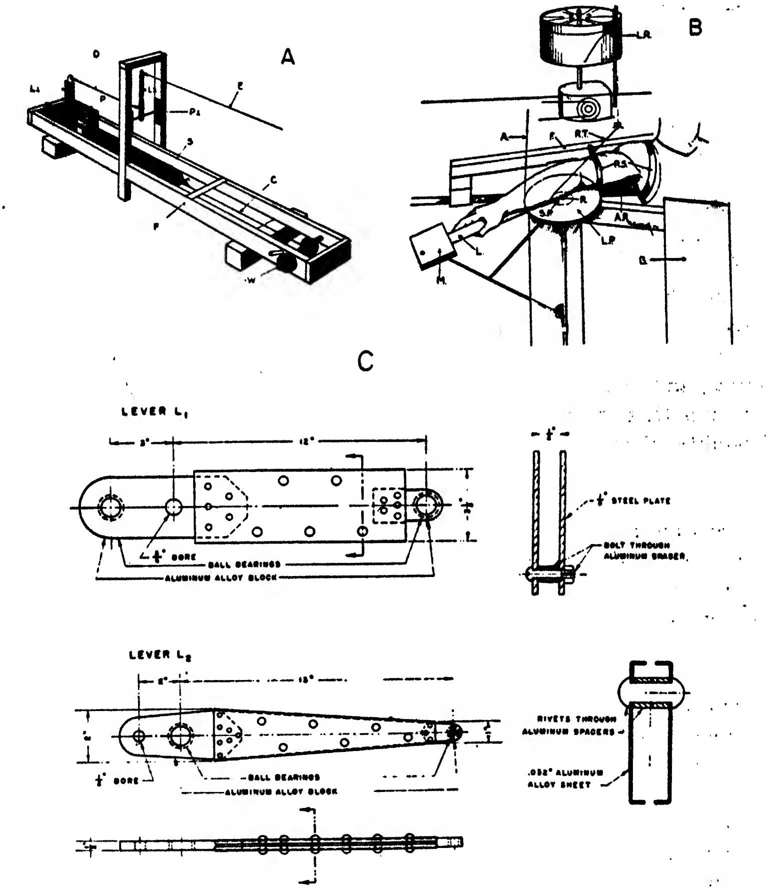

Fig. 1. Apparatus for providing a series of different loads to oppose the flexion of the arm and for recording the movement.

A. Lever system producing a series of constant forces in Wire *E*.

B. Inertia and recording lever.

C. Details of the two levers of the system shown in figure 1A. For explanation see text.

any given setting of the windlass, movement of wire E (25 cm.) by the flexion of the subject's arm elongated the spring and increased the tension in E by 0.3 kgm. The equivalent mass (inertia) of the system as a whole was practically that of the light lever and was measured to be 0.1 kgm. at wire E.

Inertia and recording lever. The subject was seated at the apparatus shown in figure 1B. It consists of a steel shaft supported perpendicularly in two hangers and carrying at its top a small and a large concentric pulley (*SP* and *LP*) and a horizontal aluminum lever (*L*). The hangers were fitted with ball bearings (thrust) to carry the shaft. Lever *L* was properly trussed to provide support for a series of added masses (*M*). After the subject was seated, the support (*F*) and arm rest (*AR*) were adjusted until the elbow joint coincided with the axis of rotation of the lever system (*R*). The subject's upper arm was fastened to the support by two leather straps (*RS*) to keep the elbow over the axis of rotation and prevent the arm from flying forward in a rapid movement. Enough restraint was provided to give good fixation of the arm on the lever while not interfering with muscular movement.

In order to produce a series of moments of inertia about the axis of rotation, or the coincident elbow joint, flat lead weights of 1 kgm. mass were added to the end of the lever. Two such weights are shown in place in figure 1B.

The maximum mass which could be carried by the lever was 16 kgm. The radius of gyration of each of the added weights was 46 cm. A segment of tire casing mounted at *B* absorbed the impact of the weights at the end of the stroke. Greater moments of inertia about the elbow joint were obtained by attaching wire *A* (figure 1B) from the periphery of the large pulley to a fly-wheel type of inertia wheel similar to that described by Hill (3). The range of moments of inertia studied was from the lowest of about 1400 kgm.-cm.^{2} (that of the arm plus the lever *L*) to 600,000 kgm.-cm.^{2}. The moment of inertia of the unloaded lever was 1000 kgm.-cm.^{2}. For the determination of isometric torques exertable at various positions of flexion of the arm, wire *A* was attached to a standard type of isometric lever.

The movement of the arm was also opposed by constant torques about the elbow joint. Wire *A* from the periphery of the large pulley was connected to wire *E* of the isotonic force system shown in figure 1A. In this way a constant force was made to act at a constant radius, resulting in a constant opposing torque. A range of torques from 0 to 300 kgm.-cm. was available.

Flexions of the arm were begun from a variety of starting positions, ranging from full extension (0°) to a position of 55° of flexion. In the following discussion, unless specified, all contractions were begun from full extension. It was found that when relaxed, the rest position of the arm was about 60° of flexion. When contraction was to be started from full extension, it was necessary to hold the relaxed arm in this position by a spring catch. This catch disengaged with the smallest increment in force. The data of the experiments described above will be presented in part I.

In part II a constant force was applied parallel to the flexor muscles. The isotonic lever system (fig. 1A) was placed 30 feet from the subject in a line with the axis of the upper arm, and wire *E* was attached to the inertia lever (*L*, fig. 1B) at a point 14.5 cm. from the axis of rotation (not shown in the fig.). Since the length of the wire was great, it remained essentially parallel to the upper arm for all positions of flexion, the lever arm of the applied force increasing according to the

sine of the angle through which the arm flexed. From the anatomical data of Braune and Fischer (6) it can be shown that the average lever arm of the forearm flexor muscles is approximately a sine function of the angle of flexion of the forearm for all positions except those below 20°. Since the lever arm of the applied force and that of the flexor muscles both vary according to the sine of the angle of flexion, this system provides an essentially constant force opposing the flexor muscles for all positions above 20°. Experiments were also done with flexion of the arm in a vertical plane (similar to the method described by Hill, 3), in which wire E from the isotonic lever system (still 30 feet distant) was attached to the wrist by a strap. The mechanics of this technique are like those described immediately above.

The moment of inertia of the forearm was determined by the method of Braune and Fischer (7) utilizing their formulae (see also Fenn, 8). The validity of these formulae has been checked by a dynamic method (8). In rapid movements a centrifugal shift of the arm on the lever will increase the effective moment of inertia somewhat, but since the magnitude of this error is not accurately known, the value estimated from the formulae will be used in subsequent calculations, with recognition of the possibility that it may be too low.

Recording. Movement was recorded as shown in figure 1B. A thread from the periphery of the small pulley (SP) was attached to a lever which wrote on a kymograph driven by a synchronous motor. The roughly parabolic displacement-time records were measured on a comparator and from small increments in displacement and time, the instantaneous velocity at a series of points along the contraction could be determined, 10 to 15 points giving adequate description of the contraction. A simpler technique, in which a smoked paper was moved past a fixed 60-cycle tuning fork by the movement of the arm, was adopted later. The distance between 60-cycle peaks is proportional to the average velocity of that interval. Both methods have comparable accuracy.

Electromyograms. Recordings were made both from skin leads and from needle electrodes in the biceps and triceps muscles. The presence of the needles did not influence the subject's mechanical performance, but movements of the electrodes at high speeds introduced technical difficulties not yet solved. Slower movements were recorded satisfactorily, and simultaneous recordings from the two muscles were made on an ink writer after suitable amplification.

Technique. The subject was instructed to relax and after a preliminary warning on the command to pull, he made a maximal voluntary flexion against the lever. In a few contractions no warning command was given and in a few others the subject was not allowed to know in advance the magnitude of the force to be used. In each case no definite difference was observed as a result of these techniques. Periodic determination of isometric tension indicated that no appreciable fatigue set in before 15 or 20 contractions.

EXPERIMENTAL

PART I. In this set of experiments, the flexion of the forearm was opposed by a moment of inertia about the elbow joint, by a constant torque or by any combination of these according to the technique described above.

(1) *Kinetics of the single contraction.* The instantaneous angular velocity (ω) of the arm at various points of flexion along a single contraction was plotted against the time (t) required for the arm to reach the respective points. The slope at any point on an ω-t curve is a measure of the instantaneous angular acceleration and, therefore, the instantaneous force. Figure 2A shows a typical

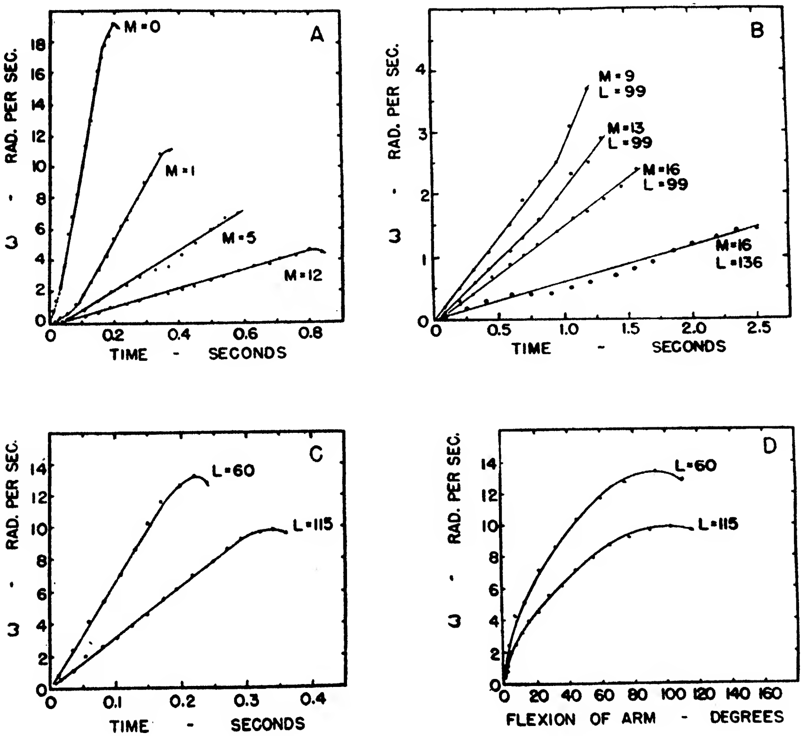

Fig. 2. Kinetics of single flexions of forearm.

A, *B* and *C*. Each curve represents the instantaneous angular velocity (ω) in radians per second developed at a series of times during a single contraction. The mass (M) in kilograms placed at the end of the inertia lever or the opposing isotonic torque (L) in kilogram-centimeters is indicated beside each line. In the experiments of figure 2A, added masses alone were used; in those of figure 2B, combinations of inertias and torques, and in 3C, a series of torques.

D. The instantaneous angular velocity plotted against the distance moved when that velocity was reached. The two contractions are those given as ω-t plots in figure 2C.

series of such plots, each line representing a single contraction against a different mass (M) placed at the end of the lever. The mass of the added weight in kilograms is indicated beside each line.

Figure 2C shows ω-t plots for single contractions against two different opposing isotonic torques. The torque (L) in kilogram-centimeters for each contraction

is also marked beside the respective line. In each of these cases there was no added mass. Figure 2B gives ω-t relationships for the various combinations of torques and added masses indicated. Figure 2D is a plot of ω against the position of the arm for each curve of figure 2C and illustrates the development of velocity with respect to the angle of flexion.

Since the slope of an ω-t plot at any point is a measure of the instantaneous angular acceleration (α), and since the moment of inertia is constant for any single contraction, a linear phase in such a plot is one of constant torque. The data of figures 2A through 2C show that the arm develops an essentially constant torque over most of the duration of a single contraction, whether the opposing force is an externally applied constant torque or the reaction of a mass.

The curves in general have three phases: *a*) an initial segment during which the slope (and, therefore, exerted torque) is increasing, *b*) a phase of constant torque occupying the greatest part of the curve and *c*) a period of decreasing slope. The first of these is demonstrated in figure 2A. The short lag (dotted line) at the beginning of the movement, where the torque is increasing to its constant value, probably represents any real delay due to nervous or muscular factors which may be present, combined with any apparent delay due to the taking up of slack in the system, particularly where the hand grasps the lever. This initial lag is present only in pure inertia experiments. In torque experiments the lever was held against the stop by the opposing applied torque (pre-loaded). No movement of the lever and recorder was possible until the arm developed a torque equal to the opposing torque; therefore, any lag which may be present is not apparent in the ω-t plots of figures 2B and 2C.

The phase of constant torque occupies the major part of the duration of a contraction. An estimation of the length of this phase in terms of position of the arm can be obtained from columns θ_L and θ_M in tables 1 and 2. Column θ_L gives the position of the arm at the last point of the linear phase of the contraction and column θ_M, the position of the arm at the point where maximum velocity is reached. Table 1 summarizes the data for a series of contractions against the moment of inertia about the elbow joint (in kgm.-cm.^{2}) given in column I. In general, these data indicate that the linear phase of contraction against inertias extends over most of the length of the movement. This implies that for any particular contraction, the torque developed is constant until most of the distance has been traversed (i.e., approximately 113° out of 120°).

In experiments where movement is opposed by a torque, the ω-t curves appear to go through a maximum (fig. 2C). Table 2 summarizes a series of contractions against the opposing isotonic torques (in kgm.-cm.) given in column L_i. Although the linear phase still occupies a large fraction of the duration of the contraction, column θ_L in table 2 shows that the average position of the arm at the end of the linear phase was 86° and the position at maximum velocity 96°. In experiments of the torque type the force exerted by the arm during the linear phase will be the sum of the applied opposing torque and the torque exerted in accelerating the mass of the forearm and lever. Since the slope of the ω-t curve begins to decrease (at an average position of 86°), the exerted torque is decreasing

beyond this position. At the maximum point on the curve (average 96°) the acceleration is zero and the exerted torque will have fallen to a value equal to the applied opposing torque. Average values of θ_L and θ_M for two other subjects were 93° and 106° for one, and 80° and 93° for the other.

Analysis of a series of contractions begun from a position of semi-flexion indicates that the absolute values of θ_L and θ_M are similar to those when the contraction started from full extension. In torque experiments θ_L and θ_M were 83° and 95°, respectively (measured from full extension as 0°), and in the inertia experiments the force was maintained to the end of the contraction.

TABLE 1. *Force-velocity data for a typical series of contractions against various moments of inertia about the elbow joint*

I kgm.-cm.^{2}	L_α kgm.-cm.	ω (80°) rad./sec.	θ_L degrees
1500	144	14.0	113
1500	144	14.2	90
4190	156	9.7	90
4190	148	9.6	113
8655	159	6.9	115
8655	142	6.6	116
24,200	153	4.2	118
24,200	153	4.1	115
102,700	176	2.1	111
102,700	145	1.8	—
320,700	154	1.1	115
320,700	138	1.1	116
612,700	125	0.8	118
612,700	119	0.8	118
Isometric (80°)	415	0.0	—
Average			113

I, in kgm.-cm.^{2}, indicates the moment of inertia; L_α, in kgm.-cm., the torque developed in accelerating I; ω (80°), in radians per second, the angular velocity attained at 80°; and θ_L, in degrees, the position of the arm at the end of the constant acceleration phase.

The precise determination of the values of θ_L and θ_M is rather difficult as will be seen from the ω-t curves presented, and this probably accounts for the wide variation in values measured. Although it is impossible at the present writing to describe accurately the equation of the single contraction, certain general conclusions seem justified. In inertia experiments it appears that the exerted force is maintained over the entire contraction, while in torque experiments it tends to decrease at mean positions of from 80° to 93° of flexion. Secondly, although the variation is large, it appears that there is no consistent association of the position of θ_L or θ_M with different values of applied torque or other variable, and that the values tend to be constant for a given subject. The estimation of the values of θ for contractions against the lightest loads (i.e., arm and lever) is the most difficult. Although of 'inertia type,' comparison of the two upper figures of tables 1 and 2

shows an extreme variation of θ. In about one half of the contractions against very light loads, the linear phase ends short and in the rest tends to extend throughout the movement. Part of the variation may be due to the difficulty in determining θ in the most rapid movements.

Another type of third phase of the single contraction is illustrated in the upper curves of figure 2B. In a very small number of contractions of over 0.8 to 1.0 second duration, an increased torque was developed toward the end of the contraction. There is nothing constant about the time or positions of the arm at which the increase occurred. The magnitude of this increase will be discussed below.

TABLE 2. *Force-velcocity data for contractions against an isotonic torque*

L_i kgm.-cm.	L_α kgm.-cm.	L_T kgm.-cm.	$\omega(80°)$ rad./sec.	θ_L degrees	θ_M degrees
0	139	139	16.1	88	97
0	138	138	16.0	100	—
60	82	149	12.1	—	—
60	86	156	12.6	70	83
82	54	136	10.3	92	104
82	61	143	11.0	85	98
110	44	154	8.7	93	104
110	32	142	7.9	85	104
135	12	147	5.8	—	—
135	21	156	6.5	88	8
152	8	160	5.8	—	—
152	11	163	5.4	100	100
64	80	145	12.0	95	—
64	65	129	11.8	95	98
91	61	152	11.1	—	—
115	39	157	7.9	72	95
145	10	155	5.2	70	90
145	10	155	4.2	—	—
173	5	177	2.9	88	88
173	4	176	2.8	100	100
186	1	187	2.4	70	90
Average				86	96

L_i, in kgm.-cm., the applied opposing torque; L_α, the torque exerted in accelerating the mass of the arm and lever; L_T, the total torque, or the sum of L_i and L_α; ω (80°), the angular velocity, in radians per second at 80°; θ_L and θ_M, in degrees, the position of the arm at the end of the linear phase and at the point of maximum velocity, respectively. The higher velocity (compare table 1) at 80° for the lightest load is due to the use of a lighter lever (700 kgm.-cm.^{2}); note that L_T is not changed.

The lowest curve of figure 2B illustrates a tendency to sinusoidal variation about the straight line assumed to represent the points. This pattern was relatively uncommon.

The data of all of the above contractions are from one subject, MH. Similar results were observed in 9 other subjects.

(2) *Force-velocity relationships.* A sample of the data of inertia-type experi-

ments on subject MH is given in table 1 in which column I is the moment of inertia about the elbow joint used for each contraction; L_α the torque developed as computed from the slope of an ω-t plot; ω (80°), the velocity attained at 80°; and θ_L is the length of the linear phase of the contraction. A position of 80° was selected for convenience since, for all subjects, the linear phase was maintained to at least this position and a more accurate determination of the slope of the ω-t plot is possible. These and similar data for MH are illustrated in the first curve of figure 3C. The velocity (ω) at 80° is expressed as a percentage of the velocity at the same position attained with the unloaded lever. The torque is expressed as a percentage of the isometric at 80°.

Data for three other subjects (initials beneath each curve) performing inertia type experiments are included in figure 3C. Four types of curve are illustrated. Subject MH tends to develop constant torque although the inertia has been increased enough to reduce the maximum velocity to 5% of that attained in the relatively unloaded contraction. The other subjects are able to develop more force with heavier loads but exhibit different patterns. Curves for four other subjects have been studied and appear to resemble that of RT. The hollow circles for subject JF represent an experiment in which the subject's eyes were covered and the weight to be used was unknown before contraction was begun. This was done since it was believed possible that greater voluntary force might be exerted when a heavier weight was expected, possibly by introducing shoulder or other muscle groups. The results were not very different with this technique. A percentage scale is used in figure 3C to illustrate the rather narrow range of forces exerted for a wide variety of velocities, and that for very light loads, the force is of the order of 30% of the isometric at 80°.

The results of the inertia experiments can be summarized in terms of the kinetics of the single contraction by figure 3D. The abscissae represent the position of the arm, and the ordinate, the total torque developed. Curve I is the isometric length-tension diagram, each solid circle indicating the average of 15 isometric contractions at the position of the arm shown. These data are from MH. Since *a*) in the single contraction constant torque is developed throughout the linear phase and *b*) approximately the same torque is developed against all of the inertias used (fig. 3C, MH), all contractions against inertias for MH may be represented by line *A* in figure 3D. The vertical line at 86° indicates θ_L and that at 100°, θ_M. In the unloaded contractions about half have a short linear phase. The torque falls off rapidly after reaching a position of about 80° (line *M*). The rest of the unloaded contractions as well as those against added masses follow line *M* to at least 113°. A second difficulty with the unloaded contractions is the precise determination of the exerted torque. Although the curve for MH in figure 3C is drawn to 37% (151 kgm.-cm.), the true average is 140 kgm.-cm. for the unloaded contraction, with a number falling as low as 120 kgm.-cm. Whether the low values represent less than maximal effort or the higher values the introduction of other muscle groups is not known. However, when an added mass of as little as 1 kgm. has been added, the exerted torque is more constant at about 150 to 160 kgm.-cm.

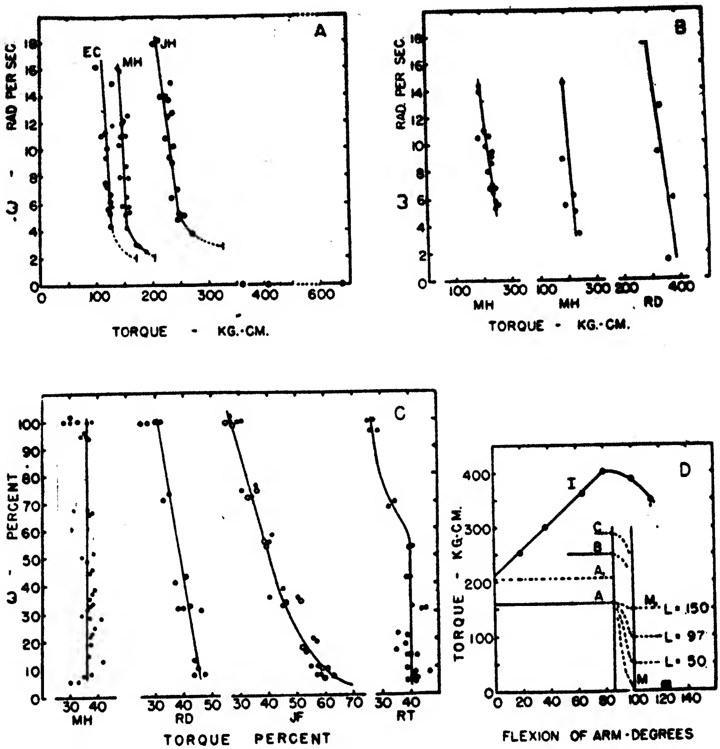

FIG. 3*A*. Force-velocity data for three subjects. Each point represents a contraction against a different opposing isotonic torque. *Ordinate:* the velocity in radians per second at 80°. *Abscissae:* the total torque in kgm.-cm. exerted by the arm. The symbols on the axis of abscissae indicate the respective isometric torques at the 80° position. The vertical line terminating the dotted extrapolation of each curve represents the value of the isometric torque at a position of full extension. The subject's initials are indicated beside each line.

B. Force-velocity data at 80° for three experiments, all contractions having a starting position of 35°. Ordinates and abscissae as for figure 3A. The first curve (subject MH) is for an experiment of the torque type; the second and third (MH and RD, respectively) are of the inertia type.

C. Force-velocity data for four subjects performing inertia type experiments. The velocity (ω) at 80° for each contraction is expressed as a percentage of the velocity at 80° attained with the lightest load. Torques exerted are expressed as a percentage of the isometric torque exertable at the 80° position. The subject's initials are indicated beneath the axis of abscissae for each curve.

D. *Abscissae:* flexion of arm in degrees. *Ordinates:* total exerted torques in kgm.-cm. (subject MH). Curve I is the isometric length-tension diagram. Lines *A*, *B* and *C* represent single contractions beginning at a position of 0, 55, and 70°, respectively. The dotted continuations of line *A* illustrate the falling off of exerted torque for inertia experiments (M_1 and *M*) for torques (*L*) of 150, 97, and 50 kgm.-cm. The vertical lines at 86° and 100° represent the end of the linear phase and point of maximum velocity, respectively, for the single contraction. The block at 120° represents the limitation of the movement by the bumper. Further description in text.

The other subjects tend to exert somewhat more force with lower velocities and, since, in their single contractions, constant torque is developed, they will be represented in figure 3D by a series of parallel lines between A and A_1.

Force-velocity curves for three subjects making contractions against various opposing torques are given in figure 3A. The data for subject MH are given in table 2. Column L_i is the applied opposing torque. Column L_α is the torque developed in accelerating the mass of the arm and lever (1200 kgm.-cm.[2] for MH). Column L_T is the total torque developed or the sum of L_i and L_α. Columns ω (80°), θ_L and θ_M represent the velocity attained, the length of the linear phase and the position of maximum velocity, respectively. Figure 3A is a plot of the velocity at 80° against the total torque (L_T) developed. The isometric torque at 80° is represented for each curve by the locus of the respective symbol on the axis of abscissae ($\omega = 0$). The curves are extrapolated toward these points. The vertical line ending the dotted extrapolations is drawn at the value of the isometric torque at the position of full extension, the starting position of the contraction. It is obvious that isotonic torques greater than the isometric at the starting position cannot be moved. The extent of the curve is not necessarily limited by this, since the abscissae represent the total torque, the sum of L_α and L_i. However, with the largest movable isotonic torques, constant torque is developed and there is little tendency to increase the L_α component. An occasional attempt to do this is indicated in the curves of figure 2B (all of low velocity), but the increase in torque is only of the order of 15% at most. It was previously shown that in contractions against isotonic torques, the exerted torque falls off between 80° and 100° to a value equal to the applied opposing torque. This is illustrated in figure 3D for three contractions by the dotted lines to L = 150, 97, and 50 kgm.

Figure 3B gives force-velocity curves at 80° for experiments in which the starting position was varied. The first curve is for a series of contractions beginning at 35° against various opposing torques by subject MH. The second curve is one of the inertia type, all contractions beginning from 35°. The third curve is one of the inertia type for subject RD, starting position 55°. The forces developed are greater than when contraction begins from full extension, but the linear phase ends at the same absolute position of the arm. This is illustrated in figure 3D by lines *B* and *C*.

Table 3 is a summary of the results from 10 subjects making a total of about 600 contractions. Experiments marked with an asterisk are of the torque θ type, while the others are inertia experiments. A number beside the subject's initials indicates in degrees the starting position of the contraction. Where there is no number contractions were made from full extension. Column L_T gives the torque exerted (at 80°) with the lightest loads. The next columns represent the isometric torque at the starting position and the isometric at 80°. L'_T and L''_T give L_T as a percentage of the starting isometric and as a percentage of isometric at 80°, respectively. The last column gives the velocity at 80° attained with the lightest load.

For the contractions beginning from full extension, the torque developed

against the lightest loads is from 26 to 38% (column L''_T) of the isometric exertable at 80°. Subjects not represented in figure 3C have curves similar to that of RT. With the greatest load used, L_T increases to a value between 37 and 50% of isometric at 80°, except JF who reaches 60%. The isometric columns of table 3 show that the exertable isometric torque at 80° is close to twice that at a position of full extension. The greater isometric torque in flexed positions (despite a shorter muscle and presumably less muscle force) is due to the greater muscle lever arm in the flexed positions. The shape of the isometric length-tension curves for all the subjects is essentially the same although on a different absolute scale. If L_T increases to 50% of isometric at 80° with heavy loads, this will represent a value of about 100% of isometric at the starting position. Since it has been argued that all subjects (including JF) develop constant torque over a single contraction, it would appear that JF exerts a torque of 120% of starting isometric with heavy loads. This apparent discrepancy is explained by the ω-t curves for this subject. With heavy loads JF has an elongated phase of increasing torque, the constant phase beginning only after some 15° have been traversed. L_T as a percentage of isometric at this position is 100. This would be equivalent in figure 3D to a curve which follows curve I to 15° of flexion and then becomes parallel to line *A*. It is obvious that for all subjects, if inertias large enough to produce an infinitely slow movement had been used, the single contraction would be represented by a curve approximating the isometric (line *I*).

For two experiments in which contraction was begun from full extension, subject MH developed torques of 71 and 67% of starting isometric (table 3). In three experiments with varying starting position the same subject developed torques of 70, 69 and 66% of starting isometric. These percentages are fairly constant although the absolute values of exerted torque vary widely. Subject RD shows a similar result, developing torques of 56 and 60% of starting isometric for contractions begun at 0° and 55°, respectively. Similar data for contractions other than unloaded show a fairly constant value of L'_T for any given subject. Table 3 shows no such constancy for comparable values of L''_T. It is suggested that L_T for any subject is a relatively constant fraction of the isometric torque that could be exerted at the particular position of starting.

Part II. In this group of experiments, the movement of the arm was opposed by a constant force applied parallel to the flexor muscles. With this technique the muscles are opposed by an approximately constant force for all angles of flexion of the arm greater than 20°. In terms of angular units the arm is opposed by a torque which increases according to the sine of the angle through which flexion occurs. The data were analyzed in angular units, since this requires no assumptions concerning muscle lever arms. Conversion to linear forces and velocities will be discussed below.

(1) *Kinetics of the single contraction.* Although only a few complete analyses are available at the present writing, certain differences in force patterns, in comparing parts I and II, are apparent. Figure 4 gives ω-t plots for five single contractions by subject JF. Opposing parallel forces were applied to the lever at a

TABLE 3. *Summary of force-velocity data for all subjects performing isotonic torque or inertia type experiments (the former are indicated by an asterisk after the subject's initials)*

SUBJECT	L_T kgm.-cm.	ISOM. START kgm.-cm.	ISOM. 80° kgm.-cm.	L'_T % start	L''_T % 80°	ω (80°) rad./sec.
MH	150	210	410	71	37	14.0
MH*†	140	210	410	67	34	16.0
EC*†	120	170	360	71	33	16.2
JH*†	210	325	640	65	33	18.0
RD	174	310	650	56	27	17.5
JF	120	220	460	55	26	14.0
RT	200	400	770	50	26	21.0
TV	175	310	570	57	31	15.0
JW	125	180	325	70	38	13.5
HB	125	150	450	83	28	15.0
MK	100	180	350	56	29	14.0
MH(55)*†	242	345	410	70	59	14.2
MH(35)*†	200	290	410	69	49	14.2
MH(35)†	190	290	410	66	46	14.2
RD(35)	240	410	650	57	37	17.5

A number in parentheses indicates the starting position of the contraction in degrees; no number indicates a start from full extension. L_T in kgm.-cm. indicates the total torque exerted with the lightest load. The next columns indicate the isometric torque at the starting position of the contraction and at 80°, respectively. Columns L'_T and L''_T give L_T as a percentage of Isom.-Start and Isom.-80°, respectively. The last column gives the velocity at 80° for the lightest load. In the experiments marked with a †, a lighter lever was used; this accounts for the somewhat higher velocity.

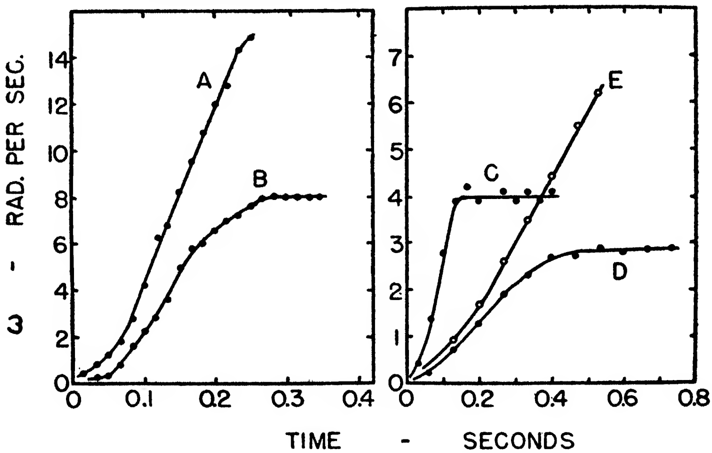

FIG. 4. Instantaneous angular velocity plotted against the time required for the arm to attain that velocity in a series of contractions in which forces parallel to the axis of the upper arm were applied at a distance of 14.5 cm. from the axis of rotation. Each line represents a single contraction against: *A*, the unloaded lever only; *B*, a parallel isotonic force of 8.7 kgm.; *C*, a parallel force of 18 kgm.; *D*, the combination of a parallel force of 18 kgm. and a 5-kgm. added mass at the end of the inertia lever; *E*, a 5-kgm. added mass alone.

distance of 14.5 cm. from the axis of rotation. The curves represent contractions against the unloaded lever (*A*), a parallel force of 8.7 kgm. (*B*), a parallel force of 18 kgm. (*C*), the combination of a parallel force of 18 kgm. and a 5-kgm. added mass at the end of the inertia lever (*D*) and the 5-kgm. mass without a parallel opposing force (*E*). Curves *A* and *E* are of the type described in part I; movement is opposed only by the reaction of an inertia about the elbow joint. In the other curves of figure 4, where parallel forces are applied, there is a marked sigmoid shape and a more prolonged constant-velocity phase terminally. All curves show an initial phase of increasing torque since early in the contraction, before the opposing torque has increased appreciably, most of the opposition to movement is the reaction of the mass of the arm and lever, and the curves will show the lag described above in connection with part I. The single contraction curves for another subject (MH) are grossly similar to these.

When the arm is opposed by various parallel forces without an added mass, the maximum velocity is reached in approximately the same time interval from the beginning of the movement for all forces (a range of 0.15 to 0.30 seconds for MH and JF). The point of maximum velocity represents very different arm positions but contractions against greater forces reach this point at progressively smaller angles. This is in contrast to the experiments of the inertia or constant torque type, in which the point of maximum velocity is associated with constant arm positions, the time intervals being variable. The effect of adding masses in the parallel force experiments is to make the curves less sigmoid and to resemble more those of the inertia type.

The torque developed at any point in these or similar contractions is the sum of the torque of acceleration and of that due to the applied opposing torque. The former can be calculated from the instantaneous slope α of the ω-t curve at any given angle $\left(\text{from } L_\alpha = \dfrac{I\alpha}{980}\right)$, and the latter from the lever arm, the applied force, and the angle ($L_i = 14.5\ F \sin\theta$). The pattern of development of force with respect to the position of the arm is illustrated in figures 5 and 6 (subjects JF and MH, respectively). This is similar to the analysis for the experiments of part I given in figure 3D.

Figure 5 is divided in two halves to avoid confusion of points. In both, abscissae represent the position of the arm in degrees and the ordinates, the total torque ($L_\alpha + L_i$) developed at any given position. Line *I* represents the isometric length-tension diagram. Line *A* (contraction *A* of figure 4) illustrates the constant torque developed in an unloaded movement and the variability of the position where exerted torque decreases. All curves are shown with an initial phase of rising torque, representing the lag in the ω-t plots. When a parallel force of 8.7 kgm. is applied, the opposing torque will rise with flexion according to $L_i = 8.7 \times 14.5 \times \sin\theta$; this is the equation of line *B*. The ω-t curve of this contraction is given in figure 4 (*B*). When the total torque ($L_\alpha + L_i$) was calculated as described above, fairly constant values were found. These are illustrated by the solid circles lying close to line *A* in the left half of figure 5. The total torque remains fairly constant, since when L_i (line *B*) increases, L_α falls off pro-

portionately. In curve *B* of figure 4, there is a terminal portion of constant velocity which begins at 70° of flexion. In this phase L_a is zero and the total torque is equal to L_i. In figure 5 it will be seen that from 70° on the solid circles fall on dotted curve *B* (i.e., L_i). In the right half of figure 5, line *A* again represents an unloaded contraction.

If a parallel force of 18 kgm. be applied, the opposing torque will rise according to dotted line *F*. A similar analysis was applied to contraction *C* (fig. 4) and the values of total torque are represented by the solid circles in the right half of

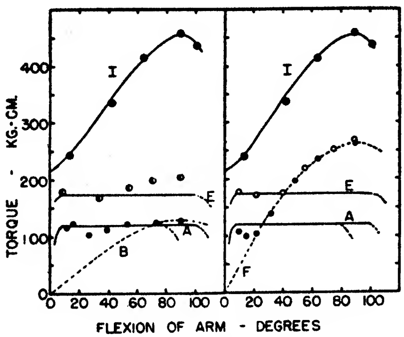

Fig. 5

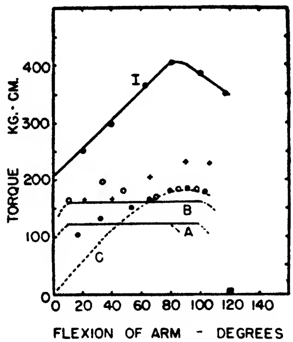

Fig. 6

Fig. 5. *Abscissae:* flexion of arm in degrees. *Ordinates:* total torque developed by the arm. Line *I* represents the isometric length-tension diagram; line *A*, a single unloaded contraction; and line *E*, a contraction against an added mass of 5 kgm. Curves *B* and *F* illustrate the rise in applied opposing torque when a parallel force of 8.7 and 18 kgm., respectively, is used. Solid circles in left side of figure represent total torque developed at a series of points in a contraction against the unloaded lever and a parallel force of 8.7 kgm.; solid circles on right side, the unloaded lever and a parallel force of 18 kgm.; hollow circles, an added mass of 5 kgm. and a parallel force of 18 kgm.; half-closed circles, an added mass of 5 kgm. and parallel force of 8.7 kgm. Subject JF.

Fig. 6. Similar to figure 5 but for subject MH. Lines *A* and *B*, respectively, represent single contractions against the unloaded lever and an added mass of 5 kgm. Curve *C* shows the rise in applied torque with a parallel force of 12 kgm. Solid circles represent a contraction against the unloaded lever and a parallel force of 12 kgm.; hollow circles, an added mass of 5 kgm. and a parallel force of 12 kgm. Crosses represent the response of JF to the same combination shown for MH by the hollow circles.

figure 5. The total torque tends to remain constant until the constant velocity phase of the single contraction is reached, after which it becomes equal to the applied opposing torque. Analysis of intermediate parallel forces has given similar results and the following working rule seems justified: the total torque developed is constant at first and remains equal to the torque that would be developed in an unloaded contraction until the applied torque rises to a value equal to the developed torque, after which the arm exerts a torque equal to the applied torque. It is apparent that the constant velocity phase is progressively longer with larger applied torques.

Line *E* in figure 5 represents a contraction in which movement is opposed by an added mass of 5 kgm. on the inertia lever (contraction *E* in fig. 4). If, in addition to the added mass, an opposing parallel torque of 18 kgm. is applied (curve *D* in fig. 4) the total torque will be represented by the hollow circles in the right half of figure 5. The results are similar to those described above; the total torque is constant and equal to the torque developed in a contraction against an added mass of 5 kgm. without an opposing parallel force, until the applied torque becomes equal to the developed torque, after which curve *F* is followed.

If, with a 5-kgm. mass on the lever, a parallel force of 8.7 kgm. is applied, the total torque will be represented by the half-covered circles in the left half of figure 5. It will be seen that the total torque follows the curve for that of a 5-kgm. mass and then follows a curve parallel to that of the applied isotonic torque but at a higher level. The kinetics of this type of contraction with combination of relatively small parallel forces and large inertias have not been studied in detail, nor has that of very small forces and the unloaded lever been investigated. It is possible, however, that if curve *B* (fig. 5) were lower, the exerted torque would not follow line *A* but could rise somewhat parallel to the curve for isotonic torque.

Figure 6 represents a similar analysis for subject MH. Line *A* represents an unloaded contraction, assuming the lower value previously discussed. The curve of L_i for an opposing parallel force of 12 kgm. is illustrated by *C*. The solid circles represent the total torque developed in such a contraction. The response is somewhat different from that of JF, the total torque being lower at the start and increasing to join the L_i curve more gradually at a higher torque level. A similar result was observed for other parallel forces. Apparently, subject MH develops less torque at first when opposed by greater loads. Line *B* represents a contraction against a 5-kgm. mass. The hollow circles give the total torque in a contraction against the combination of a 5-kgm. mass and a parallel force of 12 kgm. The torque rises at first, then falls off to join the L_i curve. The response of JF to a similar opposing load is given by the crosses in figure 6. These differences will be discussed below.

(2) *Force-velocity relationships.* Figures 7 and 8 are force-velocity curves for subjects JF and MH, respectively. In both, the ordinates represent the angular velocity, and the abscissae, the total torque developed expressed as a percentage of the isometric at 80°. The figures compare, for each subject, the force-velocity curves at 80° when the opposing loads were constant torques or inertias about the elbow joint (broken lines), and when parallel forces were used (solid lines).

In figure 7 (JF) the small solid circles and their correlation line (broken) were given on a percentage scale in figure 3C. The symbols on the solid line represent data taken from the 'parallel force' contractions analyzed immediately above: solid circle, a parallel force of 8.7 kgm., solid triangle, a parallel force of 12 kgm., and a cross, a parallel force of 18 kgm. The half-closed circle is from a contraction against an added mass of 5 kgm. and belongs to the data for experiments of the inertia type. In figure 8 (MH) the solid circles represent experiments of the 'parallel force' type. Individual points for inertia and constant torque experiments have been given on a percentage scale above (fig. 3C). The half-

closed circle represents one extreme of the curve, a contraction against a moment of inertia about the elbow joint of 600,000 kgm.-cm.[2] The hollow circle represents 80° force-velocity data for one contraction made against the combination of an added mass of 5 kgm. and a parallel force of 18 kgm.

From these data it appears that the force-velocity curves are different when different techniques of applying the opposing load are used. When combinations of parallel forces and inertias are used, the data tend to fall within the area enclosed by the two types of curve given in the figures. This is illustrated by the hollow circle in figure 8 for subject MH. Subject JF behaves similarly. The cross in figure 7 represents a contraction against a parallel force of 18 kgm. with

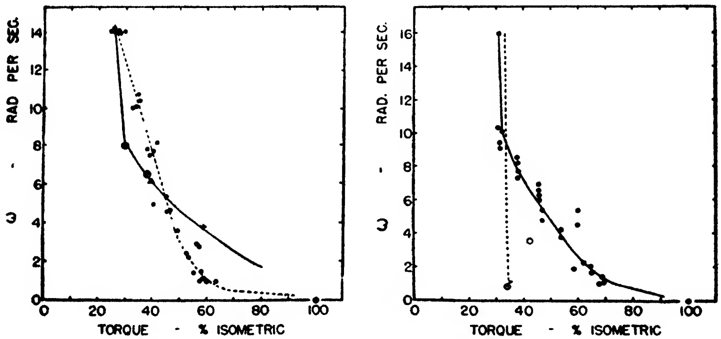

Fig. 7 **Fig. 8**

Fig. 7. Force-velocity curve for JF. *Abscissae:* total torque developed expressed as a percentage of the isometric at 80°. *Ordinates:* angular velocity at 80° (ω) in radians per second. The dotted line (through the small solid circles) is the force-velocity curve for inertia experiments previously given in figure 3C (JF). The solid line is the force velocity curve at 80° for the parallel force type of experiment. The large solid circle represents a parallel force of 8.7 kgm.; a triangle, a parallel force of 12 kgm. and a cross, one of 18 kgm.; half-closed circle, an added mass of 5 kgm.

Fig. 8. Force-velocity curve for MH. Abscissae and ordinates as for figure 7. Dotted line and solid line represent 80° force-velocity curves for torque and inertia experiments and those of parallel force type, respectively. The hollow circle represents the combination of a parallel force of 18 kgm. and an added mass of 5 kgm., and the half-closed circle, an inertia of 600,000 kgm.-cm.[2]

no added mass on the lever. The coordinates of the point are 61%, 4 rad/sec. A contraction against the same parallel force and an added mass of 5 kgm. (see data of fig. 5, right half) would be represented by a point at 61%, 3 rad/sec. This point would fall within the area between the solid and broken-line curves. Similar findings were observed with other contractions of this type.

The difference between the two types of curve might have been qualitatively predicted from the single contraction analyses of figure 5. In an unloaded contraction, line *A* is followed, and when a parallel force of 8.7 kgm. is applied, essentially the same total torque is developed. Therefore, when L_T is constant, the addition of an L_i must necessarily reduce L_α and, hence, reduce the velocity. There is a marked difference in velocity with but little change in force developed.

A few experiments have been tried in which the load is suddenly increased during the course of a contraction. It appears that if the new load is not excessive, the relatively high velocity attained with the light load is maintained for a period in spite of the increased opposition. Points obtained with this technique fall outside the force-velocity curves illustrated.

Preliminary investigation of the force-velocity curves at angles of flexion less than 80° indicated that on the same scale as for figures 7 and 8 the curves lie either within or very close to the area defined by the curves given. In plotting such curves the isometric at the particular angle selected for preparation of the curves was represented at 100% torque.

For the 'parallel force' experiments the curves for MH and JF practically coincide, in spite of the rather different patterns of torque development illustrated in figures 5 and 6. The apparent explanation is that although MH starts with less torque (and therefore less velocity) when opposed by large parallel forces, she increases the torque to a greater extent than JF before reaching constant velocity. The mean exerted torque is not very different from the constant value developed by JF.

The force-velocity data given in figure 9 are from five other subjects, each represented by a different symbol. The 'forearm vertical' technique described above was used. Subjects JF and MH are represented on this plot by the solid circles and squares, respectively. Although their data appear slightly more linear in the range of intermediate velocities, the 'forearm horizontal' technique apparently does not introduce any major differences. In this figure the abscissae represent the torque exerted at 90° expressed as a percentage of the isometric at 90°. The abscissae represent the angular velocity at 90° expressed as a percentage of the velocity at the same angle attained without an added load. A position of 90° was used since at this angle a torque-angular velocity curve is also one of linear force-linear velocity and can be more readily compared to similar data for isolated muscle. A position of 90° also represents maximum velocity. Force-velocity data taken at 80° on a percentage scale would not be distinguishable from the plot presented. For the unloaded contraction, in about one half of the experiments, the exerted torque has fallen to zero at 90°, but in most of the rest it remains high (fig. 3D). In those contractions in which it has disappeared, the curve will pass through the large solid circle at $\omega = 100$, torque $= 0\%$. However, in the cases in which it remains high, the maximum point will be at the encircled square at $\omega = 100$, torque $= 37\%$, for MH, and at $\omega = 100$, torque $= 30\%$, for JF. The variability of the maintenance of torque suggests that the falling off of exerted torque may be due to some factor other than the limitation since almost all unloaded contractions maintain full torque to this point, and the curves must pass through the encircled symbols (fig. 9). The data for the other experiments represented were obtained with the 'forearm-vertical' technique. Since the records are not suitable for detailed analysis and since it was believed originally that the force did fall in free contraction, the intimate details of the upper part of the curve for the other subjects are not available. However, it is undoubtedly true that a similar difficulty also exists for the other subjects.

The measurements for MH and JF have not been extended into the range of very low parallel forces as yet.

Table 4 summarizes the data of figure 9. For each subject the sex and physical condition (classified as athlete (*A*) or non-athlete (*N*)) are given. The isometric torque in kgm.-cm. at 90° and the velocity at 90° attained without an added load are given in the next columns. Column *Sym.* illustrates the symbol representing the subject in figure 9. Columns *b* (in cm. per second at 35 cm.

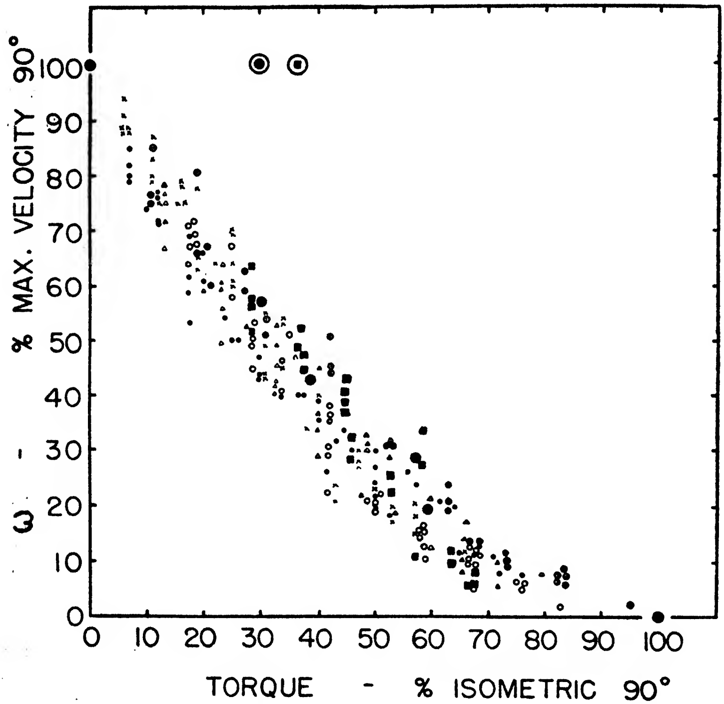

FIG. 9. Force-velocity curve at 90°. *Abscissae:* total exerted torque at 90° expressed as a percentage of isometric at 90°. *Ordinates:* angular velocity at 90° (which is also the maximum velocity) expressed as a percentage of the velocity at 90° attained with the lightest load. The encircled symbols at 100% velocity represent contractions in which the exerted force against light loads was maintained at 90° of flexion.

radius) and the ratio P_0/a, were obtained by fitting Hill's equation to the curves. It is interesting that despite the wide range of physical build as evidenced by the widely differing isometric forces, all subjects can attain approximately the same maximum velocity. This is partially accounted for by differences in the moments of inertia of the arms of the subjects. The force-velocity relationships are apparently independent of sex and physical training, the data for all subjects on a percentage scale falling on essentially the same curve.

PART III. *Electromyograms.* Simultaneous recordings with needle electrodes in both biceps and triceps muscles were made from two subjects and with surface electrodes only in five others. Results with the surface electrodes corroborate those with needle electrodes but are of themselves open to criticism. Only the isometric and inertia type of contraction has been studied to the present.

There are numerous observations in the literature on the phenomenon of contraction of antagonist muscles (9, 10). The simultaneous activity of both triceps and biceps muscles in graded and in maximal movements of the human forearm have been studied by Golla and Hettwer (11). In general, our results with maximal contractions confirm theirs. In relatively slow contractions (heavy load) there is considerable antagonist activity throughout the movement as judged by the EMG. In rapid movements technical difficulties prevent estimation of the amount of electrical activity, but that it was present is fairly certain.

TABLE 4. *Summary of the data of figure 9*

SUBJECT	SEX	PHYSICAL CONDITION	ISOM. 90° kgm.-cm.	MAX. VEL. rad./sec.	SYM.	b cm./sec.	Po/a
MKA	M	A	830	19.0	×	490	1.6
DK	M	A	750	19.0	●	325	2.0
RJ	M	N	500	18.0	⊙	280	2.3
EC	F	N	360	18.5	△	315	2.1
CT	F	N	260	19.0	○	380	1.7
MH	F	N	410	18.0	■	—	—
JF	F	N	460	15.0	●	—	—

For each subject the sex, the physical condition, (as athlete (*A*) or non-athlete (*N*)), the isometric torque at 90°, the velocity at 90° with the lightest load (*Max. Vel.*), and the symbol (*Sym.*) used in figure 9 are given. The figures of the last two columns for the constants obtained by fitting Hill's equation; *b* is expressed in cm. per second at a radius of 35 cm., and P_o/a is a ratio.

Isometric flexions of the forearm result in considerable extensor activity but less in amount than that recorded from the biceps. The triceps is more active in isometric flexions at 90° than when the arm is in more extended positions. A few recordings of isometric extension show that for at least one subject (MH) the biceps (here the antagonist) remains relatively inactive electrically.

DISCUSSION

(1) *The significance of the force-velocity curve.* The data presented in this paper describe the relationship between force and velocity in maximal flexions of the forearm. Since the electromyograms indicate activity of antagonist muscles in all contractions, the measured forces are resultants and, therefore, are lower than the true tension in the agonist. If it could be shown that the antagonists develop a constant (or measurable) tension in all contractions, and that a maximal voluntary flexion produces a maximal (or constant percentage of maximal)

tetanizing stimulus to the forearm flexors, the observed force-velocity curves for arm movements could be compared to those for isolated muscle. Certain features of the mechanical records suggest, however, that the innervation is variable. In general, it has been shown that different curves relating force to velocity are obtained with different techniques of application of the opposing load.

The true force-velocity curve of human (isolated) muscle must lie outside the curves presented. The true isometric tension is greater than our measured value since it has been shown that there is considerable antagonist activity. The maximal unloaded velocity can be estimated from other data if it is assumed that all mammalian muscles are similar. Fenn and Marsh (12) have shown that the maximal velocity in cat quadriceps is about 5 cm/cm. muscle length/sec. from extrapolation of their curves. If then the human biceps is 16 mm. long, it should have a maximal velocity of about 80 cm /sec. If the mean lever arm of the flexor muscles is 3 cm., this would represent an angular velocity of 26 radians per second. In figure 9 this point would be $\omega = 160\ \%$, torque $= 0$. What the real value of the isometric torque would be if there were no antagonist activity cannot be estimated from data available at present. However, the true force-velocity curve must pass through some point greater than the measured isometric and through a maximum velocity of the order of 160% of the measured maximum. The exact course of the curve is not determinable from simple force-velocity measurements in the intact arm.

Therefore, although the curves of figures 7 and 8 can obviously not be represented by an equation of the form given by Hill (1) there is no evidence that his equation may not be applicable to human (isolated) muscle. Curiously enough, the individual subject's data (fig. 9) can be rather accurately represented by a rectangular hyperbola. The constants for each given in the last columns of table 4 have obviously no fundamental significance, since the curves do not represent the total exerted torque in the range of high-velocity, the total torque curve passing through the encircled symbols.

(2) *Method of study of human muscle.* Fenn, Brody and Petrilli (13) reported a series of quick release experiments in which force and velocity were measured over a time interval after release which was short enough to preclude the interference of reflex modification of the movement. With this technique a given velocity increase results in a smaller loss of tension than when release is not used. This method has been criticized on the grounds that the elastic component of muscle, which is presumably not affected by movement velocity, is stretched by the contractile component, and at the time of release the former is responsible for the smaller coefficient of tension loss. While this criticism is partially justified for a single release, from the point of view of pure mechanics the quick release method is theoretically more acceptable than 'free swings.' The behavior of a purely elastic element is independent of the load but is dependent on the initial tension before release. If a muscle in which there is no co-activity in the antagonist be selected, the isometric tension is a determinable quantity. On

release against a series of opposing loads any relationship between the velocity and the force developed in the earliest part of the contraction must necessarily be one of muscular properties. After a release both the contractile and elastic elements will be active in producing movement. Although it is possible that in practice the latter may partially obscure the contractile component, any drop in force associated with higher velocity is either a property of the contractile machine or a frictional factor. This applies equally well to isolated muscle. If force-velocity relationships do not agree with those obtained with the conventional method of study of isolated muscle, i.e., in relatively 'steady-state' movements, it must be argued that the properties of the machine are different under various conditions of contraction and that equations of motion for muscle must be specific for a particular set of mechanical conditions. It would still be necessary to select a muscle group where co-contraction of the antagonists would not be prominent, possibly the extensors of the forearm in certain subjects.

SUMMARY

1. An apparatus is described with which it is possible to produce a series of inertias, torques and linear forces of adequate magnitude to explore the range of forces capable of being exerted by human subjects.

2. Voluntary maximal flexions of the forearm were made against opposing loads, applied in three ways: *a*) moments of inertia, *b*) constant torques about the elbow joint as a center of rotation, and *c*) constant linear forces parallel to the axis of the upper arm (equivalent to a torque which increases as the arm flexes).

3. The kinetics of the single contraction were studied. During the first part of the movement the three techniques give similar results—an essentially constant torque is developed. In the last part of the movement the pattern of movement is different for each method of applying the load.

4. The force-velocity curves at 80° of flexion for contractions against inertias and constant torques are similar for a given subject but are different from the curves obtained with techniques using increasing torques. The results are independent of the sex, muscular development and training of the subject.

5. Electromyograms recorded from the biceps and triceps in general corroborate findings previously reported. There is considerable activity in the antagonists in isometric flexions and in contractions in which movement occurs.

6. The significance of force-velocity data in arm movements is discussed. It is concluded that such data are not necessarily a measure of force-velocity relationships in human (isolated) muscle. Hence Hill's equation cannot be fairly tested by simple measurements of limb movements.

7. A possible method of study of the mechanics of human muscle is suggested.

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AVAILABLE AND INTERSTITIAL FLUID VOLUMES OF NORMAL CHILDREN[1]

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Received for publication July 14, 1947

Although the changes in the volumes of the tissue fluids in disease are well-known phenomena in children, little is known concerning this volume in health. The report of Robinow and Hamilton (1) describes the only experimental determination of the extracellular fluid volumes of children of the white race recorded in the literature. The present investigation was undertaken to supplement this knowledge. Together with the determination of the blood volume (2) it represents one phase of a comprehensive study of physical fitness in children which is being conducted in this laboratory.

Sixty-five children varying in age from 3 to 17 years were studied. The younger children were of both sexes. They were selected from patients at the Country Home for Convalescent Children as representing physiologically normal states at the time the determinations were made. The 54 children over 10 years of age were normal, healthy boys[3] who came to the laboratory for the sole purpose of taking physical-fitness tests. Determinations of the fluid volumes were made while the child was in the resting, fasting state.

The method used was essentially that described by Gregersen and Stewart (3) for the simultaneous determination of the blood and extracellular fluid volumes. The number of blood samples was reduced to a minimum, 4 samples being drawn at half-hour intervals after the injection of the thiocyanate and dye solutions in addition to the control sample. Usually the thiocyanate had reached a constant level in the plasma of the last two samples. In a few cases there was no proof that a constant value had been reached in two hours. In such cases the two-hour concentration was used in calculating the thiocyanate space.

Thiocyanate concentrations in the plasma were determined by the technic described by Forbes, Dill and Hall (4) and read in the Evelyn photoelectric colorimeter using filter 490. A calibration curve was constructed from thiocyanate-free plasma filtrates containing amounts of trichloro-acetic acid equivalent to that in the unknown and aliquots of a solution containing 1 ml. of the the original 5% sodium thiocyanate solution in 500 ml. of distilled water.

The 'available fluid' volume was calculated as recommended by Gregersen and Stewart (3) by dividing the milligrams of thiocyanate injected by the milligrams of thiocyanate per liter of plasma. It corresponds to 'Space A' as used

[1] This work has been conducted under a grant from the Douglas Smith Foundation at the University of Chicago.

[2] Deceased March 8, 1944.

[3] We are indebted to many friends and to the University of Chicago Settlement House, the Hyde Park Neighborhood House and the Valentine Boy's Club for sending the boys to us.

by Stewart and Rourke (5) and by Kaltreider, Meneely, Allen and Bale (6). Interstitial fluid volumes were calculated to correspond to the data of Forbes, Dill and Hall (4) by subtracting the plasma volume and 70% of the red cell volume from the available fluid volume. While it is recognized that the interstitial fluid volume so measured does not represent a precise fluid compartment of the body, it is believed that it represents a fairly definite entity which has physiological significance.

The results are given in table 1. Figure 1 shows the relations of the available fluid volume to the age, height, weight and surface area of the child. The relations of the interstitial fluid volumes to the same physical measurements are quite similar but with more scattering of the individual data. Those curves relating the available fluid volume to height, weight and surface area have been fitted by the method of least squares. The curves fit the data fairly well. The relationship of the available fluid volume to body weight throughout childhood and adolescence may be expressed by a linear equation, to stature by a semilogarithmic equation and to surface area by an equation of the second degree.

The curves relating the fluid volumes to height and surface area are not quite satisfactory in that they do not bend sharply enough to take in a group of points representing boys of 9 to 11 years, with approximate heights of 140 cm. and surface areas of 1.1 square meters. Although more suitable single curves have not been found, it is possible to fit the plotted data for both height and surface area by a combination of two straight lines meeting at a point in the group of 9- to 11-year old boys. For purposes of expressing these linear relationships for limited age groups, the children have been divided into two groups representing surface areas of 0.5 to 1.2 and 1.0 to 2.0 square meters, respectively. The linear equations which have been derived for the two groups by the method of least squares are as follows:

For children with surface areas of 0.5 – 1.2 sq. m.:

Available fluid volume = −6.14 + 0.1042 height

" " " −1.48 + 9.12 surface area

Interstitial fluid volume = −3.40 + 0.0674 height

" " " −0.44 + 5.95 surface area

For children with surface areas of 1.0 – 2.0 sq. m.:

Available fluid volume = −27.20 + 0.2557 height

" " " −7.15 + 14.22 surface area

Interstitial fluid volume = −20.10 + 0.1883 height

" " " −5.31 + 10.46 surface area

Useful equations which cover the whole range of childhood and adolescence relate the fluid volume to weight:

Available fluid volume = −0.03 + 0.287 weight

Interstitial fluid volume = +0.02 + 0.209 weight

The 'a' constants of the equations are so small that they may be neglected.

Table 2 gives the standard errors for predictions based upon both the comprehensive equations covering the age range from 3 to 17 years and the linear equa-

TABLE 1

CASE NO.	SEX	AGE	HEIGHT	WEIGHT	SURFACE	AVAILABLE FLUID VOLUME	INTERSTITIAL FLUID VOLUME
6	M	3.33	103.5	17.9	0.70	5.28	4.18
7	F	4.17	95.0	12.3	0.57	3.57	2.68
8	F	4.33	109.5	18.5	0.75	4.83	3.68
9	F	4.92	103.6	15.6	0.67	4.46	3.19
10	M	5.00	106.2	16.5	0.70	4.92	3.75
12	M	6.58	121.5	21.3	0.85	6.45	5.08
14	M	6.92	127.0	26.0	0.96	8.42	6.40
15	M	7.50	121.4	21.6	0.86	6.45	4.98
16	M	7.92	121.5	25.0	0.91	7.54	5.44
17	M	8.25	121.3	19.0	0.81	5.34	3.83
20	M	8.67	137.1	30.6	1.09	8.70	6.22
21	M	8.83	120.7	22.3	0.86	6.43	4.79
22	M	8.92	139.2	28.9	1.08	8.22	5.76
23	F	9.17	136.0	30.6	1.08	7.30	5.17
24	M	10.08	136.1	25.4	1.00	7.48	5.33
25	M	10.33	139.0	29.0	1.08	7.76	5.21
26	M	10.50	146.6	38.2	1.26	10.72	7.87
29	M	11.17	142.7	28.8	1.09	8.70	5.88
30	M	11.33	138.0	28.9	1.07	8.42	6.11
31	M	11.50	142.5	32.4	1.14	8.97	6.54
33	M	12.33	139.7	32.0	1.11	8.72	6.41
34	M	12.42	150.8	36.5	1.26	11.00	8.13
35	M	12.42	149.2	36.6	1.25	10.26	7.55
36	M	12.50	151.4	38.5	1.29	11.54	8.87
37	M	12.58	147.6	38.0	1.26	9.54	6.99
38	M	12.92	154.2	40.4	1.33	11.70	8.66
39	M	13.00	147.3	39.1	1.28	10.07	7.16
40	M	13.17	155.5	43.6	1.38	12.52	9.20
41	M	13.25	145.2	35.9	1.21	10.52	7.63
42	M	13.42	151.0	39.9	1.31	12.68	9.40
43	M	13.67	151.7	40.1	1.32	11.20	8.45
44	M	13.67	156.5	47.2	1.44	12.95	9.98
45	M	13.75	164.2	45.6	1.47	12.03	8.46
46	M	13.92	170.1	49.9	1.56	15.84	12.84
47	M	14.00	156.0	36.7	1.29	10.05	7.46
48	M	14.08	152.5	40.8	1.33	11.27	8.19
49	M	14.08	163.3	60.3	1.65	18.24	14.56
50	M	14.08	168.7	57.9	1.66	16.11	11.42
51	M	14.17	153.7	48.1	1.43	13.73	9.63
52	M	14.25	150.0	43.3	1.35	12.73	9.23

TABLE 1—*Continued*

CASE NO.	SEX	AGE	HEIGHT	WEIGHT	SURFACE	AVAILABLE FLUID VOLUME	INTERSTITIAL FLUID VOLUME
53	M	14.33	177.0	62.2	1.77	19.04	14.16
54	M	14.33	169.4	54.4	1.62	16.55	12.72
55	M	14.33	166.1	44.9	1.47	13.50	10.17
56	M	14.67	154.4	45.3	1.40	12.20	8.85
57	M	14.75	167.7	58.4	1.66	15.30	11.05
58	M	14.75	182.7	76.7	1.98	19.74	13.53
59	M	14.83	150.1	36.2	1.25	10.89	8.02
60	M	14.83	169.8	57.8	1.66	17.44	12.81
61	M	14.83	173.6	63.1	1.76	18.95	14.03
62	M	15.17	170.1	51.8	1.59	15.05	11.10
63	M	15.33	185.4	68.3	1.90	18.20	13.21
64	M	15.42	177.3	60.7	1.76	17.54	12.64
65	M	15.50	172.9	61.6	1.73	18.40	13.83
66	M	15.50	179.4	58.4	1.74	17.70	12.79
67	M	15.67	178.7	62.5	1.79	19.79	14.48
68	M	15.67	174.8	57.9	1.70	16.08	11.65
69	M	15.92	162.2	48.9	1.50	15.04	10.91
70	M	15.92	162.0	45.5	1.45	12.69	8.84
71	M	16.08	172.0	56.5	1.66	17.54	12.83
72	M	16.17	175.1	69.0	1.84	19.74	14.84
73	M	16.42	172.2	64.6	1.76	16.45	12.06
74	M	16.50	177.2	61.2	1.76	17.60	12.87
75	M	16.75	175.5	59.9	1.74	17.35	12.77
76	M	16.83	182.6	70.3	1.90	18.45	12.52
77	M	17.00	167.3	55.9	1.62	18.10	13.14

tions covering age ranges of approximately 3 to 12 years and 12 to 17 years. If the choice of the best standard of reference is to depend upon the one giving the smallest standard error, body weight would be chosen for the younger group and surface area for the older group. In corresponding data for adults, surface area has been chosen as the standard of reference giving the least amount of scatter (4, 7–9).

In a study of the blood volume it was found by statistical analysis that a combination of two standards of reference, height and chest girth, serves better than height, weight or surface area alone. A similar analysis of the relation of the available and interstitial fluid volumes to various combinations of anthropometric measurements has not proved very fruitful. The standard errors for predictions based upon combinations of two standards of reference are given in table 3. In the younger group the error of prediction is least when the volumes are calculated from height and the index of build. In the older group no combination of standards of reference has given so close a prediction as surface area alone.

Whatever standards of reference are taken, the available fluid volumes show less variation than the interstitial fluid volumes. The variation measured by $\pm$ 2 standard errors represents $\pm$ 13% of the predicted values for available fluid and a corresponding $\pm$ 18% for interstitial fluid, when predictions are based upon those standards of reference giving the least variation.

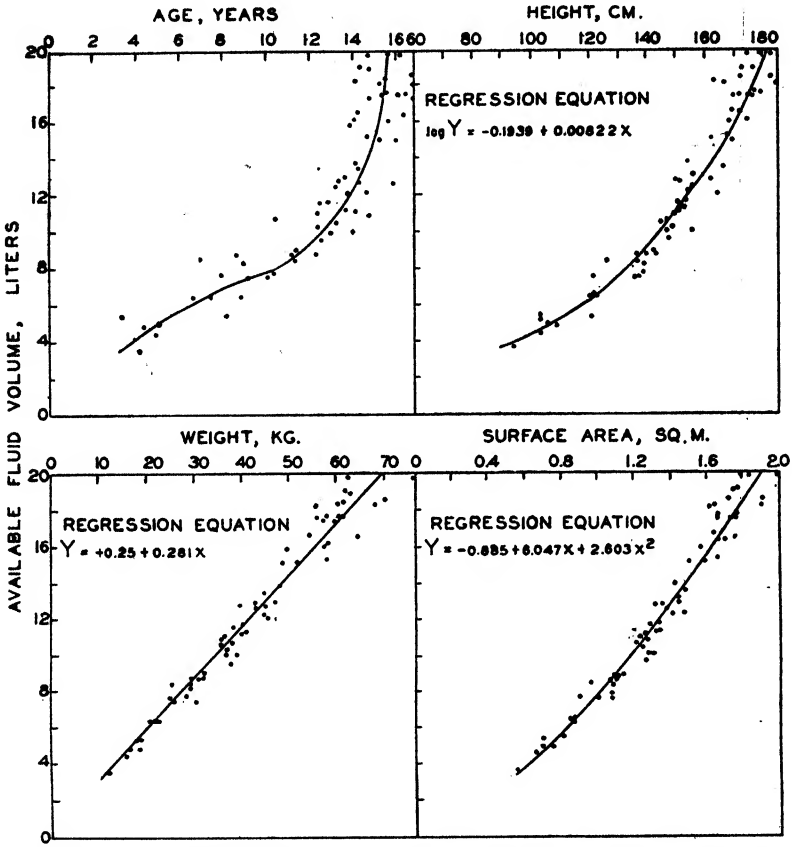

FIG. 1. RELATION OF THE AVAILABLE FLUID VOLUMES OF NORMAL CHILDREN to their age, height, weight and surface. The lines representing the relation to height, weight and surface area were fitted to the data by the method of least squares.

The question of the relationship of the fluid volumes to the state of nutrition of the child was investigated by relating the deviations from the predicted volumes to the index of weight of the child. If the predicted values were based upon surface area the deviations showed no trend related to the index of weight. Surface area is dependent to a certain degree upon the state of nutrition and is probably a poor standard on which to base such a comparison. In the report of

the blood volume (2) it was suggested that the height and chest girth describe approximately the skeletal framework of the child and that predictions based upon these standards of reference are independent of the composition of the substance surrounding that framework. This conception was applied to the inter-

Table 2. *Comparison of height, weight and surface area as standards of reference for predicting the available and interstitial fluid volumes of normal children*

STANDARDS OF REFERENCE	SIZE OF THE CHILD IN TERMS OF SURFACE AREA			
	0.5–1.2 sq. m.	1.0–2.0 sq. m.	0.5–1.2 sq. m.	1.0–2.0 sq. m.
	Number of Cases			
	20	54	20	54
	Standard Error of Estimate[1]			
	Liters		% of the Mean	
For Available Fluid Volume				
a. Predictions based upon the comprehensive equation for children of all sizes				
Height	0.71	1.22	10.3	8.9
Weight	0.52	0.96	7.5	7.0
Surface area	0.62	0.97	9.0	7.1
b. Predictions based upon the linear equation for children in the range of surface areas specified				
Height	0.60	1.22	8.7	8.9
Surface area	0.51	0.91	7.4	6.7
For Interstitial Fluid Volume				
a. Predictions based upon the comprehensive equation for children of all sizes				
Height	0.76	1.35	15.1	13.5
Weight	0.51	0.95	10.1	9.5
Surface area	0.66	0.97	12.4	9.7
b. Predictions based upon the linear equation for children in the range of surface areas specified				
Height	0.55	1.08	10.8	10.8
Surface area	0.49	0.91	9.7	9.1

[1] Standard Error of Estimate $= \sqrt{\frac{\Sigma\Delta^2}{DF}}$ where Δ represents the deviation of the volume found from that predicted from the appropriate regression equation, and DF the degrees of freedom, $n-2$ in the case of height and weight, $n-3$ in the case of surface area.

stitial fluid volumes by plotting the deviations from the values predicted by height and chest girth as frequency distributions for groups representing various weight indices. Approximately equal numbers of positive and negative deviations were found in all of the groups. The lack of relationship of the inter-

stitial fluid volume to the state of nutrition, which such a distribution of the deviations suggests, finds support in the report of Keys (10) that the available fluid volumes of young adults showed no significant change as their weight was reduced 24% by starvation.

In spite of the fact that body weight is a comparatively poor standard of reference because of the variable substance which it represents, it serves as a useful

TABLE 3. *Comparison of single and combinations of anthropometric measurements as standards of reference for the prediction of the available fluid and interstitial fluid volumes of normal children*

STANDARDS OF REFERENCE	SIZE OF THE CHILD IN TERMS OF SURFACE AREA			
	0.5–1.15 sq. m.	1.0–2.0 sq. m.	0.5–1.15 sq. m.	1.0–2.0 sq. m.
	Number of Cases			
	20	54	20	54
	Standard Error of Estimate			
	Liters		% of the Mean	
For Available Fluid Volume				
Height	0.60	1.22	8.7	8.9
Weight	0.49	0.96	7.0	7.0
Surface area	0.51	0.91	7.4	6.7
Height and weight	0.49	0.92	7.1	6.7
Height and index of build	0.43	1.00	6.3	7.3
Height and chest girth	0.46	0.97	6.7	7.1
Weight and index of weight	0.49	0.92	7.1	6.7
For Interstitial Fluid Volume				
Height	0.55	1.08	10.8	10.8
Weight	0.44	0.95	8.8	9.5
Surface area	0.49	0.91	9.7	9.1
Height and weight	0.46	0.92	9.1	9.2
Height and index of build	0.42	0.93	8.4	9.3
Height and chest girth	0.46	0.95	9.1	9.5
Weight and index of weight	0.46	0.93	9.1	9.3

basis for comparing the relative effect of the age of the child upon the volumes of the various fluid compartments. In figure 2 the blood, plasma, available and interstitial fluid volumes per kilogram of body weight are plotted against the age of the child. The chart also shows the relation of age to the packed cell volume and to the ratio between the interstitial fluid and the blood volumes. While the blood volume increased from an average of 80 ml. per kilogram of body weight at 3 years to 90 ml. per kilogram at 16 years, the available fluid volume per unit of body weight remains quite constant at 287 ml. per kilogram. The

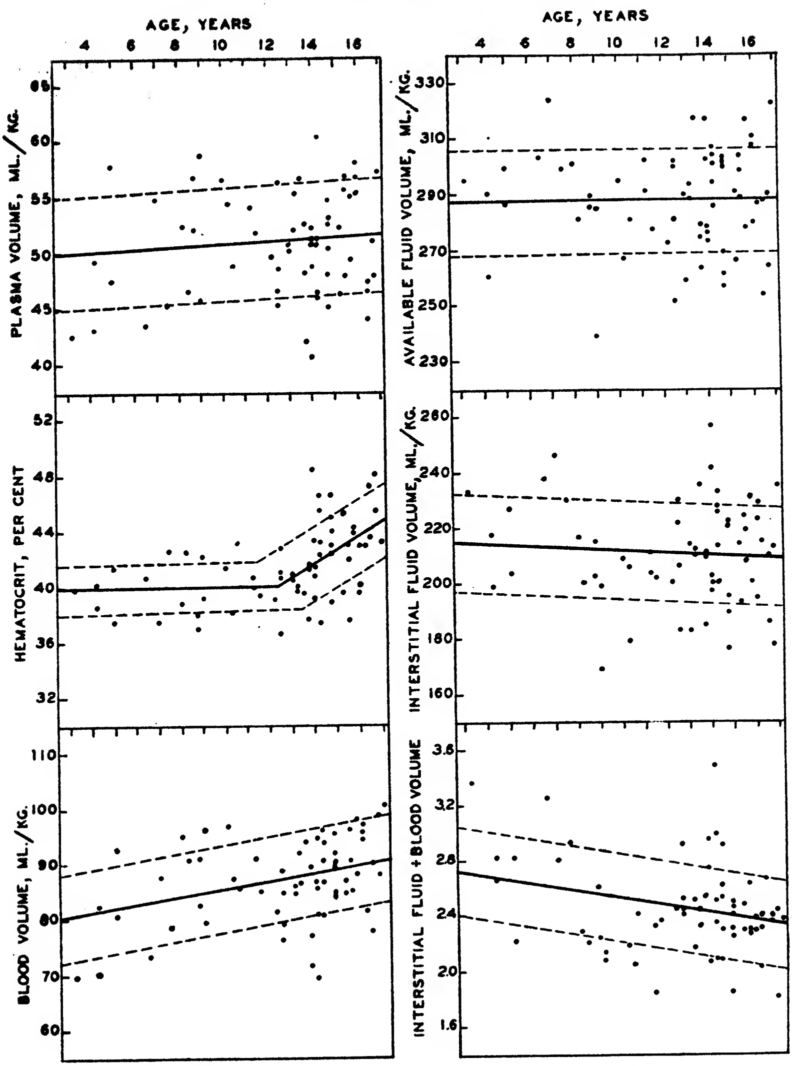

FIG. 2. RELATION OF THE BLOOD AND TISSUE FLUID VOLUMES PER UNIT OF BODY WEIGHT to the age of the child. The solid lines represent trends as calculated by the method of least squares, the broken lines the range of variation represented by ± 1 standard error of estimate from the trend lines.

Certain inconsistencies have been introduced by representing the trend lines for the unit plasma and blood volumes by single straight lines and that for the hematocrit by two straight lines. It is also possible that variations with growth and development have been masked by the use of the single straight lines. Better internal consistency and perhaps better agreement with the facts could be obtained by representing the trends by averages for age groups. It does not seem that such an elaboration is justified at present in view of the comparatively few children studied in the younger age groups and the wide variation among individuals of a given age group.

slight upward trend for the plasma volumes and downward trend for the interstitial fluid volumes are of questionable significance, but a tendency toward smaller interstitial fluid volumes in boys of 9 to 11 years of age which is reflected in a decrease in the ratio of the interstitial fluid to the blood volume suggests some change in the body fluids of possible physiological significance during that period of childhood.

DISCUSSION

The literature contains little information on the available fluid volumes of children. The values of Robinow and Hamilton (1), all above 300 ml/kgm., are on the whole higher than those of the present study which average 287 ml/kgm. The discrepancy can probably be explained by the fact that the majority of children studied by these authors were 10 to 20% underweight. Our values for white children are similar to those of a group of negro boys of an earlier study (11) if the volumes are related to surface area, but are slightly lower when referred to body weight. The available fluid volume of the negro boys averaged 299 ml/kgm.[4] The volumes per liter of body surface reported by Forbes, Dill and Hall (4) for 4 negro children in Mississippi agree with our values for white children of the same age.

Values in the literature for the available fluid volume of adults (5–7, 9, 12) vary quite widely with reported averages from 191 to 257 ml/kgm. Values for 4 young male adults studied in this laboratory averaged 235 ml/kgm., with variations from 177 to 289 ml/kgm. It seems reasonably certain that the available fluid volume of the child and adolescent exceeds that of the adult when referred to a unit of body weight. Although the various investigators agree that surface area shows closer correlation with the available fluid volume than does body weight, it is difficult to compare children with adults on this basis, for our results have shown that the ratio of the available fluid volume to surface area increases with age. The 16- and 17-year-old boys of our study had an average available fluid volume of 10.14 l/sq. m. as compared with average values varying from 7.19 to 9.7 l/sq. m. for adults as reported in the literature.

SUMMARY

The available fluid volumes of 65 normal children varying in age from 3 to 17 years were calculated from the dilution of intravenously injected sodium thiocyanate. The interstitial fluid volume was calculated by subtracting the plasma volume and 70% of the red cell volume from the available fluid volume.

The available fluid volumes were found to be related to body weight by a linear equation, to stature by a semilogarithmic equation and to surface area by an equation of the second degree. Equations of similar form were found to describe the relationship of the interstitial fluid volume to height, weight and surface area. In addition to comprehensive equations covering the whole range of

[4] Values as originally reported were lower because the plasma volume had been subtracted.

childhood and adolescence, linear equations covering limited age groups were calculated to relate both available and interstitial fluid volumes to stature and to surface area. Two such equations cover the age range from 3 to 17 years in each case.

Judged by the standard error of estimate, surface area was found to be the best standard of reference for the available and interstitial fluid volumes of the child. For the younger child a combination of height and index of build as standards of reference gave the least variation from the predicted values of any of the standards of reference tested. For the older boys the use of surface area as the standard of reference gave closer predictions than combinations of anthropometric measurements.

Unlike the blood volume, the interstitial fluid volume was found not to vary with the state of nutrition of the child.

The available fluid volume per unit of body weight was found to remain constant throughout the age range of 3 to 17 years. A tendency toward smaller interstitial fluid volumes noted in boys of 9 to 11 years of age which was reflected in a decrease in the ratio of the interstitial fluid to the blood volume suggests some change in the body fluids of possible physiological significance during that period of childhood.

The authors wish to express their appreciation to Dr. D. B. Dill of the Fatigue Laboratory at Harvard University whose cooperation instigated this and other problems relating to the physical fitness of the child.

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BLOOD VOLUMES OF NORMAL CHILDREN[1]

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Received for publication July 14, 1947

The introduction by Gibson and Evans (1) of the blue dye technic for the determination of plasma volume made possible more accurate values than could be secured by using the red dyes and stimulated further investigation into the subject of blood volume. The present study used this technic to determine basic values for the blood volume of normal children of all ages which could serve for comparison with children whose blood volume is abnormal.

Seventy-seven normal children varying in age from 1 to 17 years were studied. The 23 children under 10 years of age were critically selected by the physician-in-charge from patients at Bobs Roberts Memorial Hospital and at the Country Home for Convalescent Children as representing physiologically normal states at the time the blood volume determinations were made. They represented both sexes. All of the children 10 years of age or older were normal, healthy boys[3] who came to the hospital for the sole purpose of taking tests for physical fitness. The children were in the resting, fasting state at the time the blood samples were drawn for the determination.

The method used was essentially the modification of the blue dye technic described by Gregersen and Stewart (2) for the simultaneous determination of plasma volume and thiocyanate space. Dye concentrations in the plasma were determined with the Evelyn photoelectric colorimeter (3). The logarithmic character of the disappearance slope as described by Gregersen and Rawson (4) was taken into account in calculating the initial dilution of the dye by the plasma. The total blood volume was calculated from the plasma volume by means of the relative packed cell volume. No correction was made for the volume of plasma trapped in the cells, since it was desired to compare the blood volumes with earlier values described in the literature in which this correction had not been made. Such a correction would lower the reported volumes by approximately 4% (5).

To allow for variations in size, the height and weight of each child was recorded and the surface area calculated from the formula of DuBois (6) or the nomogram (7) based upon that formula. For many of the children additional measurements of chest girth, hip width, knee width and subcutaneous fat gave material for estimating body type and nutritive status by calculation of the index of build and index of weight as described by McCloy (8). It was hoped that

[1] This work has been conducted under a grant from the Douglas Smith Foundation at the University of Chicago.

[2] Deceased March 8, 1944.

[3] We are indebted to many friends and to the University of Chicago Settlement House, the Hyde Park Neighborhood House and the Valentine Boy's Club for sending the boys to us.

such information might facilitate the analysis of individual variations in blood volume and serve as a basis for more accurate predictions of the normal blood volume of a given child.

The results are given in table 1. Figure 1 shows the relation of the blood volumes to body height, weight, surface area and age. With the exception of the curve relating the blood volume to the age of the child, the lines drawn through the plotted points are based upon equations which have been determined by statistical analysis to fit the whole range of height, weight or surface covered by childhood and adolescence. The relation of blood volume to body weight is represented by a linear equation, to body surface by a second degree equation and to stature by an exponential function which is fitted best by the Gompertz curve.

The standard errors of estimate of table 2 show the comparative value of each of these measurements as standards of reference for predicting the blood volume of a given child. The smaller the standard error of estimate, the smaller will be the range of variation around the trendline represented by the regression equation. Judged by the error of estimate, a closer prediction can be made from surface area than from either height or weight alone. By dividing the data into three groups determined by the size of the child, specifically by his surface area, it is shown that the error of estimate increases with the size of the child but that its value relative to the predicted volume (per cent) varies little from early childhood through adolescence, especially when the prediction is based upon surface area.

Within each of the three groups representing surface areas of 0.4–0.8, 0.8–1.4 and 1.4–2.0 square meters, respectively, the relation of blood volume to height, weight or surface area is practically linear. Linear equations have been derived by the method of least squares to express these relationships.

For children with surface areas of 0.4–0.8 sq.m.:

Blood volume = +0.3 + 77.14 weight
" " = −706.1 + 19.05 height
" " = −268.8 + 2267 surface area

For children with surface areas of 0.8–1.4 sq.m.:

Blood volume = +41.3 + 85.23 weight
" " = −4305 + 50.62 height
" " = −1173 + 3511 surface area

For boys with surface areas of 1.4–2.0 sq.m.:

Blood volume = +214.3 + 92.66 weight
" " = −9835 + 87.55 height
" " = −3339 + 5074 surface area

For clinical purposes linear equations have the advantage of simplicity. The standard errors of estimate based upon these equations are shown in table 2. A comparison with the errors of estimate based upon the comprehensive equations indicates that accuracy of prediction of blood volume is not impaired appreciably in any case and in some instances is improved by calculating from linear equations which cover a limited range of body size. In any size group the equa-

TABLE 1

CASE NO.	SEX	AGE	HEIGHT	WEIGHT	SURFACE	CHEST GIRTH	McCLOY RATING: INDEX OF BUILD	McCLOY RATING: INDEX OF WEIGHT	HEMATOCRIT READING	PLASMA VOLUME	BLOOD VOLUME
		yr.	cm.	kgm.	sq. m.	cm.			%	cc.	cc.
1	M	0.75	68.0	9.8	0.41	—	—	—	36.8	426	674
2	F	1.17	73.5	8.1	0.41	—	—	—	40.3	340	570
3	M	1.17	79.0	10.5	0.47	—	—	—	42.2	449	778
4	M	2.08	82.5	10.4	0.48	—	—	—	36.3	544	854
5	M	2.17	85.3	14.0	0.55	—	—	—	41.3	597	1017
6	M	3.33	103.5	17.9	0.70	50.2	0.964	1.080	39.6	753	1246
7	F	4.17	95.0	12.3	0.57	46.5	0.834	0.998	40.4	604	1013
8	F	4.33	109.5	18.5	0.75	49.7	0.913	1.114	38.5	798	1298
9	F	4.92	103.6	15.6	0.67	51.2	0.940	0.988	37.5	899	1438
10	M	5.00	106.2	16.5	0.70	52.9	1.029	0.906	41.3	782	1330
11	F	5.00	102.0	14.5	0.64	—	—	—	39.0	744	1220
12	M	6.58	121.5	21.3	0.85	53.8	0.910	1.027	40.8	926	1565
13	F	6.67	120.6	23.7	0.89	—	—	—	41.4	970	1656
14	M	6.92	127.0	26.0	0.96	61.0	1.020	1.008	37.4	1423	2274
15	M	7.50	121.4	21.6	0.86	53.5	0.946	1.003	42.6	976	1700
16	M	7.92	121.5	25.0	0.91	59.0	1.071	1.014	38.8	1457	2381
17	M	8.25	121.3	19.0	0.81	53.8	0.850	0.982	42.6	993	1730
18	M	8.33	123.0	23.3	0.89	—	—	—	41.0	1275	2160
19	F	8.50	121.9	23.0	0.88	—	—	—	42.4	1067	1852
20	M	8.67	137.1	30.6	1.09	61.3	1.001	0.999	37.9	1735	2795
21	M	8.83	120.7	22.3	0.86	57.0	0.918	1.075	37.0	1160	1842
22	M	8.92	139.2	28.9	1.08	60.1	0.960	0.968	39.1	1695	2785
23	F	9.17	136.0	30.6	1.08	61.2	0.992	1.015	42.3	1404	2432
24	M	10.08	136.1	25.4	1.00	58.3	0.867	0.983	41.4	1434	2450
25	M	10.33	139.0	29.0	1.08	61.0	0.943	0.976	38.2	1571	2540
26	M	10.50	146.6	38.2	1.26	67.3	1.066	0.986	43.2	1858	3273
27	M	10.58	136.0	30.1	1.08	—	—	—	40.0	1416	2363
28	F	11.00	143.0	30.2	1.11	—	—	—	41.9	1444	2490
29	M	11.17	142.7	28.8	1.09	62.4	0.940	0.902	40.0	1925	3210
30	M	11.33	138.0	28.9	1.07	61.2	0.974	0.961	40.7	1560	2630
31	M	11.50	142.5	32.4	1.14	64.4	1.010	0.951	39.3	1673	2760
32	M	11.58	142.0	30.6	1.11	—	—	—	44.8	1663	3012
33	M	12.33	139.7	32.0	1.11	65.0	1.019	0.956	39.0	1596	2619
34	M	12.42	150.8	36.5	1.26	67.1	0.979	0.947	36.6	2047	3230
35	M	12.42	149.2	36.6	1.25	65.3	0.972	0.985	42.8	1780	3110
36	M	12.50	151.4	38.5	1.29	66.7	1.000	0.969	41.1	1793	3047
37	M	12.58	147.6	38.0	1.26	67.9	1.045	0.974	40.8	1725	2912
38	M	12.92	154.2	40.4	1.33	69.0	0.960	1.000	40.9	2050	3470
39	M	13.00	147.3	39.1	1.28	69.1	1.057	0.997	40.7	1962	3310
40	M	13.17	155.5	43.6	1.38	70.5	1.015	0.997	39.9	2267	3770

TABLE 1—*Continued*

CASE NO.	SEX	AGE	HEIGHT	WEIGHT	SURFACE	CHEST GIRTH	McCLOY RATING INDEX OF BUILD	McCLOY RATING INDEX OF WEIGHT	HEMATOCRIT READING	PLASMA VOLUME	BLOOD VOLUME
		yr.	*cm.*	*kgm.*	*sq. m.*	*cm.*			%	*cc.*	*cc.*
41	M	13.25	145.2	35.9	1.21	68.7	1.066	0.938	39.9	1977	3290
42	M	13.42	151.0	39.9	1.31	69.6	1.022	0.983	39.4	2258	3725
43	M	13.67	151.7	40.1	1.32	68.6	0.967	1.025	37.6	1933	3100
44	M	13.67	156.5	47.2	1.44	70.6	1.051	1.035	41.4	1985	3390
45	M	13.75	164.2	45.6	1.47	67.8	0.878	1.026	41.2	2398	4080
46	M	13.92	170.1	49.9	1.56	80.5	1.029	0.872	40.7	2566	4330
47	M	14.00	156.0	36.7	1.29	70.1	0.981	0.852	43.4	1685	2980
48	M	14.08	152.5	40.8	1.33	69.3	1.007	0.983	39.0	2130	3491
49	M	14.08	163.3	60.3	1.65	81.3	1.165	1.034	41.3	2461	4195
50	M	14.08	168.7	57.9	1.66	80.0	1.065	1.000	48.5	2823	5486
51	M	14.17	153.7	48.1	1.43	76.9	1.177	0.966	37.3	2892	4620
52	M	14.25	150.0	43.3	1.35	74.3	1.120	0.982	45.5	2208	4055
53	M	14.33	177.0	62.2	1.77	80.2	1.030	0.984	43.1	3191	5610
54	M	14.33	169.4	54.4	1.62	76.1	0.963	1.026	42.3	2530	4390
55	M	14.33	166.1	44.9	1.47	72.8	0.957	0.902	46.7	2065	3875
56	M	14.67	154.4	45.3	1.40	71.3	1.094	0.961	43.0	2190	3845
57	M	14.75	167.7	58.4	1.66	79.8	1.081	0.990	46.7	2631	4940
58	M	14.75	182.7	70.3	1.90	96.1	1.130	1.016	45.0	4027	7320
59	M	14.83	150.1	36.2	1.25	66.6	0.958	0.962	38.9	1987	3255
60	M	14.83	169.8	57.8	1.66	81.5	1.011	1.014	42.4	3058	5310
61	M	14.83	173.6	63.1	1.76	79.8	1.043	1.023	43.9	3183	5670
62	M	15.17	170.1	51.8	1.59	77.8	1.019	0.895	39.5	2708	4480
63	M	15.33	185.4	68.3	1.90	81.5	0.973	1.022	45.2	3164	5770
64	M	15.42	177.3	60.7	1.76	86.6	1.041	0.936	37.5	3450	5520
65	M	15.50	172.9	61.6	1.73	82.3	1.030	0.994	42.9	2990	5240
66	M	15.50	179.4	58.4	1.74	76.3	0.939	0.962	42.0	3257	5610
67	M	15.67	178.7	62.5	1.79	85.2	0.997	0.980	43.9	3438	6120
68	M	15.67	174.8	57.9	1.70	76.7	0.968	0.977	44.3	2844	5110
69	M	15.92	162.2	48.9	1.50	78.8	1.081	0.894	43.0	2703	4745
70	M	15.92	162.0	45.5	1.45	71.9	0.913	0.988	39.4	2643	4360
71	M	16.08	172.0	56.5	1.66	77.4	0.957	1.010	40.1	3210	5360
72	M	16.17	175.1	69.0	1.84	88.9	1.146	0.967	43.0	3208	5630
73	M	16.42	172.2	64.6	1.76	81.6	1.013	1.065	43.5	2850	5045
74	M	16.50	177.2	61.2	1.76	83.7	1.095	0.888	47.3	2900	5510
75	M	16.75	175.5	59.9	1.74	83.0	0.999	0.942	45.5	2875	5280
76	M	16.83	182.6	70.3	1.90	87.5	1.065	0.978	48.2	3589	6930
77	M	17.00	167.3	55.9	1.62	80.1	1.030	0.950	43.2	3193	5620

tion relating to body surface gives a closer prediction of blood volume than those relating to weight or height.

Although the plasma volume decreases and the cell volume increases in relation to the total blood volume as the child grows, the relation of the plasma volume to body height, weight and surface area appears quite similar to that of

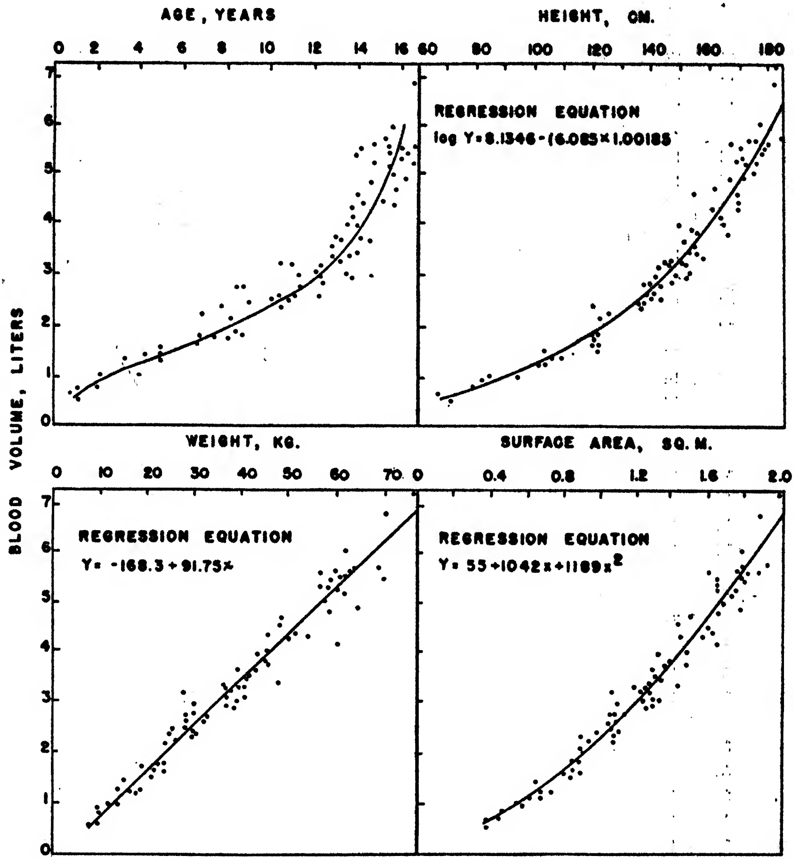

FIG. 1. RELATION OF THE BLOOD VOLUMES OF NORMAL CHILDREN to their age, height, weight and surface area. The line representing the trend with age was drawn freehand. The lines representing the relation to height, weight and surface area were fitted to the data by the method of least squares.

the blood volume. For a given size range these relationships are fairly linear and can be expressed by regression equations.

For children with surface areas of 0.4 – 0.8 sq.m.:

Plasma volume = +1.8 + 46.61 weight
" " = −806.6 + 15.69 height
" " = −162.6 + 1374 surface area

For children with surface areas of 0.8 – 1.4 sq.m.:

Plasma volume = +63.2 + 49.40 weight
" " = −2486 + 29.56 height
" " = −650 + 2044 surface area

TABLE 2. *Comparison of height, weight and surface area as standards of reference for predicting the blood and plasma volumes of normal children*

STANDARDS OF REFERENCE	SIZE OF THE CHILD IN TERMS OF SURFACE AREA					
	0.4–0.8 sq. m.	0.8–1.4 sq. m.	1.4–2.0 sq. m.	0.4–0.8 sq. m.	0.8–1.4 sq. m.	1.4–2.0 sq. m.
	Number of Cases					
	11	37	30	11	37	30
	Standard Error of Estimate[1]					
	Milliliters			% of the Mean		
For Total Blood Volume						
a. Predictions based upon the comprehensive equation for children of all sizes						
Height	111	283	489	10.6	10.2	9.6
Weight	142	245	478	13.6	8.8	9.3
Surface area	86	239	420	8.2	8.6	8.2
b. Predictions based upon the linear equation for children in the range of surface areas specified						
Height	94	275	520	9.0	9.9	10.2
Weight	119	238	443	11.4	8.5	8.7
Surface area	89	235	427	8.6	8.4	8.4
For Plasma Volume						
Predictions based upon the linear equation for children in the range of surface areas specified						
Height	65	167	302	10.3	10.1	10.4
Weight	89	153	274	12.7	9.2	9.4
Surface area	63	148	268	10.0	8.9	9.2

[1] Standard Error of Estimate = $\sqrt{\frac{\Sigma\Delta^2}{DF}}$ where Δ represents the deviation of the volume found from that predicted from the appropriate regression equation, and DF the degrees of freedom, N−2 in the case of height and weight, N−3 in the case of surface area.

For boys with surface areas of 1.4 – 2.0 sq.m.:

Plasma volume = +330.1 + 44.72 weight
" " = −4350 + 42.47 height
" " = −1192 + 2457 surface area

The standard errors of estimate of the plasma volume for the three groups of children of sizes represented by 0.4–0.8, 0.8–1.4 and 1.4–2.0 square meters of body surface are given in table 2. They are slightly higher than the corresponding values for total blood volume. As in the case of blood volume, the most

accurate predictions of plasma are derived from the surface area of the child rather than from his height or weight alone.

Without doubt the blood volume of a child is determined to a great degree by his size. Since almost any study of additional factors or conditions affecting the blood volume depends upon comparing children who vary greatly in size, the question of selecting standards which best represent the size of the child is an important one. For this reason the value of combinations of anthropometric measurements as standards of reference for predicting the blood volume

TABLE 3. *Comparison of single and combinations of anthropometric measurements as standards of reference for the prediction of the blood volumes of normal children*

STANDARDS OF REFERENCE	SIZE OF THE CHILD IN TERMS OF SURFACE AREA			
	0.8–1.4 sq. m.	1.4–2.0 sq. m.	0.8–1.4 sq. m.	1.4–2.0 sq. m.
	Number of Cases			
	31	30	31	30
	Standard Error of Estimate			
	Milliliters		% of the Mean	
For Plasma Volume				
Height	173	302	10.0	10.4
Weight	155	274	9.0	9.4
Surface area	149	268	8.7	9.2
Height and weight	149	267	8.7	9.2
Height and index of build	148	264	8.6	9.1
Height and chest girth	145	248	8.5	8.5
Weight and index of weight	150	269	8.7	9.3
For Total Blood Volume				
Height	281	520	9.7	10.2
Weight	231	443	8.0	8.7
Surface area	234	427	8.1	8.4
Height and weight	230	424	7.9	8.3
Height and index of build	225	452	7.8	8.8
Height and chest girth	223	412	7.7	8.1
Weight and index of weight	218	423	7.6	8.3

was investigated by statistical methods. Table 3 compares the standard errors of estimate of those combinations which have been found to give the more significant further increase in accuracy of prediction. The combinations chosen give a better estimate of the shape or composition of the body than do height, weight or surface area alone. Weight and the index of weight take into account the element of subcutaneous fat. Height and the index of build or height and chest girth differentiate the slender child from the one who is large boned and stockily built. They represent much of both the vertical and the horizontal elements of the body.

Since the calculation of multiple regression equations from correlation coefficients assumes a linear relationship between the variables, it was necessary in using height as a variable to limit the children in a given analysis to those whose blood volumes show a linear relation to height. The two groups with surface areas of 0.8–1.4 and 1.4–2.0 square meters, respectively, satisfy this condition. Judged by the standard errors of estimate, the closest prediction of blood volume is obtained in the younger group from weight and the index of weight. With this exception the combination of height and chest girth appears to give the most accurate prediction of both blood and plasma volume.

The relation of the blood and plasma volumes to height and chest girth may be expressed by the following equations.

For children with surface areas of 0.8 – 1.4 sq.m.:

$$\text{Plasma volume} = -2473 + 40.62 \text{ chest girth} + 11.15 \text{ height}$$

$$\text{Blood volume} = -4382 + 78.85 \text{ chest girth} + 15.51 \text{ height}$$

For boys with surface areas of 1.4–2.0 sq.m.:

$$\text{Plasma volume} = -4134 + 41.29 \text{ chest girth} + 21.90 \text{ height}$$

$$\text{Blood volume} = -9440 + 75.52 \text{ chest girth} + 49.94 \text{ height}$$

Within the limits of $\pm$ 2 standard errors of estimate the blood volumes of normal children may be expected to vary within approximately $\pm$ 16% of the values calculated from these equations. The corresponding range of variation of plasma volumes represents $\pm$ 17% of the predicted values. Eighty % of the blood volumes and 72% of the plasma volumes measured did not deviate from the predicted volumes by more than $\pm$ 10%. Two children showed deviations from the predicted blood volumes which have exceeded $\pm$ 2 standard errors of estimate. Three children showed similarly large deviations from the predicted plasma volumes.

The deviations of the blood and plasma volumes from those predicted by height and chest girth have been plotted in figure 2 as frequency distributions for the various age groups. When the calculation is based upon the equation appropriate for the size of the child, as in the left side of the chart, the distributions show no significant variation that might be attributed to the age of the child. The right side of the chart, however, shows that the equation which fits children of surface areas of 0.8–1.4 square meters gives values that are too low when applied to boys of larger size. A comparison of the equations shows that the regression coefficient for height is relatively great in the equation for the larger boys. In the calculation of the predicted volumes the value of the component measured by chest girth is less than that measured by height in the larger boys, while the reverse is true for the smaller children. The greater weight placed upon the longitudinal element of the body as a factor in determining the blood volume during adolescence suggests that the increase of the blood volume at that time is influenced by a cross-sectional factor related directly to stature. A development of the musculature during that period would conceivably represent such a factor.

A further analysis of the data, based upon deviations of the blood volume

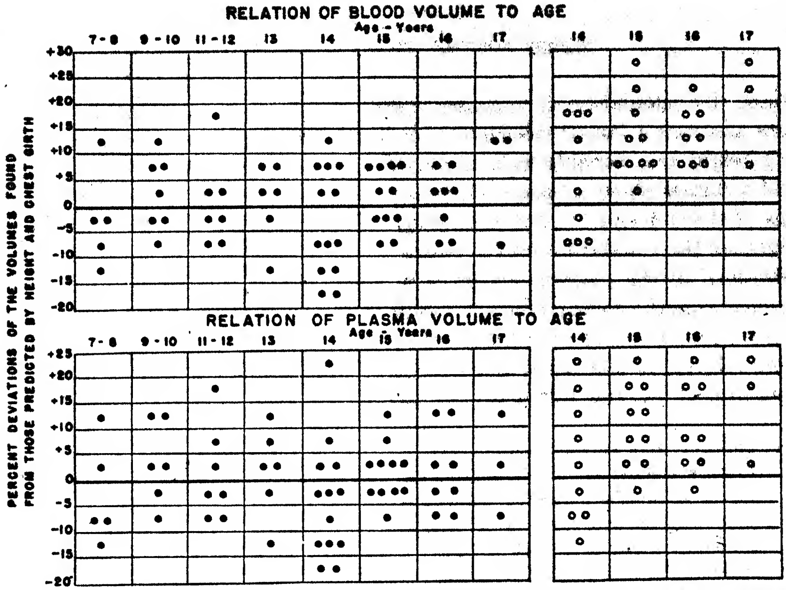

FIG. 2. RELATION OF AGE to blood volume as shown by the distribution of the deviations of the blood and plasma volumes found from those predicted by height and chest girth. ● = Predictions based upon the equation appropriate for the size of the child. ○ = Predictions based upon the equations for smaller and younger children, surface area 0.8 — 1.4 sq.m.

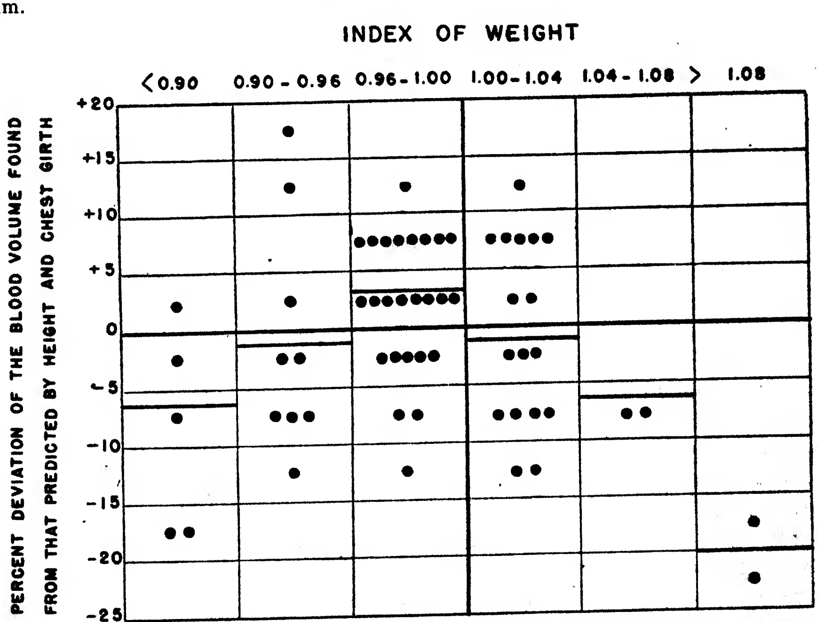

FIG. 3. EFFECT OF THE STATE OF NUTRITION, as measured by the index of weight (8), upon the blood volume of the child. The children have been grouped according to their index of weight and the deviations of the blood volumes found from those predicted by height and chest girth charted as frequency distributions for each group. The horizontal bars represent the mean for each group. The two obese boys, weight index > 1.08, have not been included in the statistical analysis.

from the values predicted from height and chest girth, has given evidence that the blood volume is related to the state of nutrition of the child. This analysis is based upon the conception that height and chest girth[4] measurements will describe a skeletal size and that variations from the blood volumes predicted from these standards of reference can be used therefore to determine to what extent the blood volume is affected by the composition of the body around the framework, in other words, by the state of nutrition.

The frequency distributions of the deviations from the predicted volumes for groups of children representing different weight indices and presumably different states of nutrition are shown in figure 3. The distribution indicates that children in a good state of nutrition tend to have larger blood volumes for their skeletal size than do either underweight or obese children. It is also of interest that the group with weight indices just below the normal average of 1.0 shows a great majority of deviations above the predicted values, while the group with weight indices just above the normal average shows about equal distribution above and below the predicted values. The chances that the differences between these two groups are statistically significant are approximately 9 to 10. A moderate degree of leanness would seem to favor the development of a large blood volume, but leanness to the point of gross undernutrition has the opposite effect.

DISCUSSION

Several earlier reports in the literature have described the blood volume of normal children (9–15). In most cases plasma volume was determined by using a red dye and a short mixing time, and the blood volume calculated from the plasma volume and the hematocrit reading. In one case the CO-inhalation method was used (9). One of the reports (15) describes values derived by the method of the present study, using the blue dye T-1824, and calculating the dye concentration from a disappearance curve as described by Gibson and Evans (1). Two of the reports come from the United States, the others from Europe.

With two exceptions (12, 15) body weight is the only measurement given to describe the size of the child. A study of these reports raises doubts as to the absolute accuracy of any of the blood volume methods. The relation of blood volume to body weight differs in the various reports, even with the same methods. To what extent the results have been influenced by racial, nutritional and hematopoietic factors cannot be determined by the data available. Our results suggest that if surface area or height and chest girth had been used as standards of reference instead of weight the discrepancies between the various reports would be reduced. It is discouraging to find that the blood volumes determined in our laboratory tend to be higher than those found by Brines, Gibson and Kunkel (15) when referred to either height, weight or surface area in spite of the fact that the technic of Gibson and Evans was used in both laboratories.

In the hands of a given investigator the results of the blood volume determination by the dye technic are usually consistent and are valuable for comparative purposes.

[4] Chest girth measurements were corrected for subcutaneous fat as recommended by McCloy (8).

SUMMARY

The plasma volumes of 77 normal children varying in age from 1 to 17 years were determined by the blue dye technic of Gibson and Evans. Their total blood volumes were calculated from the plasma volume and hematocrit reading.

The blood volumes were found to be related to body weight by a linear equation, to stature by an exponential equation representing the Gompertz curve and to surface area by a non-linear equation of the second degree. Less variation from the regression curve was found by using surface area as the standard of reference.

For the sake of convenience the relations of the blood and plasma volumes to height and surface area were represented also by linear equations representing limited ranges of surface area, 0.4–0.8, 0.8–1.4 and 1.4–2.0 square meters. Accuracy of prediction were not impaired by using the appropriate linear equation.

The value of various combinations of anthropometric measurements as standards of reference for predicting the blood and plasma volumes was investigated. With one exception the greatest accuracy of prediction came from multiple regression equations relating the blood and plasma volume to the height and chest girth of the child. Two equations were necessary to represent the range from 7 to 17 years, one for children from 7 to 14, the other for boys from 14 to 17 years. With the appropriate equation, deviations from the predicted volumes showed no significant variation with age.

Using height and chest girth as standards of reference the blood volume was found to be related to the state of nutrition of the child as measured by his index of weight. The results suggest that a moderate degree of leanness favors a large blood volume but that both leanness to the point of gross undernutrition and obesity have the opposite effect.

The authors wish to express their appreciation to Dr. D. B. Dill of the Fatigue Laboratory at Harvard University whose cooperation instigated this and other problems relating to the physical fitness of the child.

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A ROENTGENOGRAPHIC STUDY OF THE EFFECT OF A PNEUMATIC ANTI-BLACKOUT SUIT ON THE HYDROSTATIC COLUMNS IN MAN EXPOSED TO POSITIVE RADIAL ACCELERATION

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Received for publication May 16, 1947

The symptoms of blackout and unconsciousness resulting from exposure to positive radial acceleration have been attributed to reduction in the circulation in the head as a result of insufficient arterial blood pressure at head level. Two factors which might be responsible for reduction in pressure in the cerebral and ocular circulation are *a*) an increase in the pressure required at heart level to elevate blood to the head due to the increase in effective weight of the blood, and *b*) reduction in the blood pressure at heart level resulting from impaired venous return from below the heart, particularly from the splanchnic reservoir. Pneumatic anti-blackout suits have been devised which raise the blood pressure at head level by an amount sufficient to increase man's tolerance to positive g. It has been shown that the most important feature of these suits is the application of pressure to the anterior abdominal wall. The purpose of this study was to examine by means of roentgenograms the changes in the distance from the base of the heart to the base of the skull, in the height of the column of abdominal organs, and in the intrarectal pressure produced during exposure to positive g with and without pressurization of Navy Z-2 (Army G-4) anti-blackout suits.

METHODS

This series of experiments was conducted on the human centrifuge at the University of Southern California (see ref. 1, p. 330). In the cab of the centrifuge a standard aircraft seat was mounted. Casette holders with par speed screens, accommodating x-ray plates measuring 14 x 36 inches, were placed against the back of the seat. Due to the unusual length of these x-ray plates, it was possible to obtain roentgenograms of the subjects extending from the top of the head to the buttocks. A small portable x-ray machine (General Electric, model F, type 4) was mounted on the front end of the cab of the centrifuge at a distance of 1.2 meters from the casette. The roentgenograms were exposed using the full capacity of the x-ray tube, 25 milliamperes at 65 kilovolts with exposures varying from 1 to $1\frac{1}{4}$ seconds. The total exposure of any one subject was less than 30 R units. The timing unit, which activated the x-ray tube, was

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[2] The views expressed do not necessarily represent those of the Navy Department.

mounted on the side of the cab so that the subjects could initiate the exposure of each roentgenogram at the proper phase of respiration.

The measurement of intrarectal pressure was accomplished by means of balloons fastened to rubber catheters (#8F) which were connected to a simple fluid manometer. The balloons were found to accommodate between 8 and 10 cc. of air without an increase in internal pressure. In use, the balloons were inserted well into the ampulla of the rectum, which had been previously evacuated. The position of the balloon within the rectum was judged from the amount of catheter remaining outside the anal sphincter. A reference point consisting of a piece of lead was fastened to the skin with adhesive tape at the right mid-axillary line a distance of 10 inches (25.4 cm.) above the level of the balloon in the rectum. The vertical distance from the balloon to the highest point on the right leaf of the diaphragm was determined using this reference point. The need for such a precaution stemmed from the fact that penetration of x-ray required to allow visualization of the rectal balloon itself would necessitate extended exposure times and would preclude measurement of the intrarectal pressures by the method to be described.

The free end of the catheter was connected to a simple fluid manometer by means of vinyl tubing through a T-tube which was used to inflate the balloon by injecting air into the system. The manometer was filled with a saline solution having a specific gravity of 1.055 to approximate that of blood. This solution was not radiopaque so a small airtight capsule was fashioned from lucite with a lead disc inserted into the airchamber. The position of the lead disc was easily detected on the roentgenograms. It was found that the buoyancy of the capsule supported the lead disc 1 cm. below the level of the column of fluid in the manometer tube.

A horizontal bar was fastened to the back of the seat by means of a bracket. This bar extended over the right shoulder of the subject and served to support the manometer tube by its upper end, so that its long axis remained parallel to the direction of action of the accelerative force. A lucite scale having lead strips at 1-cm. intervals was fastened to the manometer tube.

The rectal balloon was inflated with considerable care so that the quantity of air could be retained at a minimum. After the balloon had been unfolded by repeated inflations, a measured quantity of air was injected into the system by means of a large syringe. As mentioned in previous reports the rectal balloon does not inflate until the pressure in the manometer exceeds the intrarectal pressure. For this reason no fluctuation in the position of the meniscus could be produced by straining until the column of fluid in the manometer exceeded an average level of 34 cm. After the column of fluid reached this height, it was possible to insert an additional 5 to 7 cc. of air without influencing the level of pressure sustained at the end of expiration, if the balloon had become adequately unfolded. Under these conditions the pressure in the manometer responded promptly to changes in intra-abdominal pressure associated with respiratory activity, speaking or straining. The syringe was removed and this leg of the T connection was plugged.

Since each subject was required to expose the roentgenograms during the application of radial acceleration, the manometer was held before him during several preliminary runs to acquaint him with the response of the intrarectal pressure to positive g and respiration with and without inflation of the anti-g suit. This procedure also graphically demonstrated the necessity for voluntary relaxation during the runs to allow reproducible results.

Each subject was fitted with a coverall type anti-blackout suit (Navy Z-2, Army G-4). The pressurizing valve was set at 1.2 pounds per g for all runs. Calibration of this valve revealed that it was actually delivering 1.18 pounds per g.

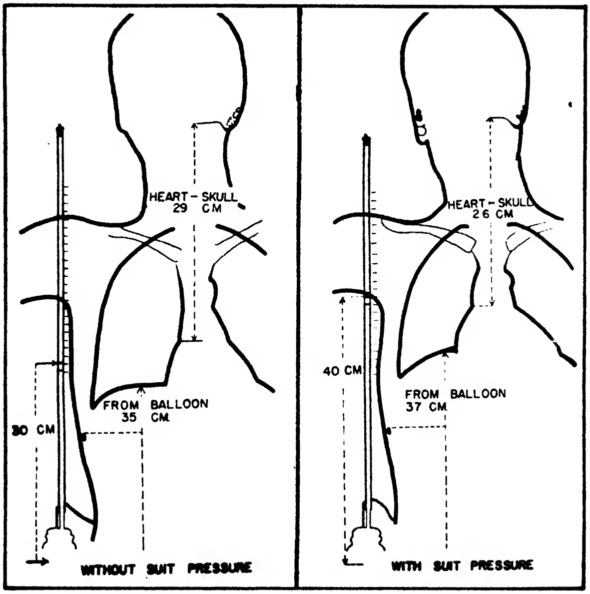

FIG. 1. TRACINGS OF THE PERTINENT CONTOURS ON ROENTGENOGRAMS taken at the end of a 'normal expiration' during the application of 5 g with and without pressurization of a pneumatic anti-blackout suit. The numerical values represent the average of 6 subjects.

Three of the subjects used in the experiment were members of the Laboratory staff and 3 additional subjects were students who had experience in numerous previous studies on the centrifuge. Five x-rays were taken without pressurization of the suit at the end of a 'normal' expiration with voluntary relaxation at the following levels of acceleration: 1 g, 2.5 g, 3 g, 4 g and 5 g. Each of these runs was repeated under the same conditions except that the suit was pressurized. Since the suit did not apply pressure below 2.5 g, manual control of the valve was used to apply one pound of pressure in the suit at 1 g. At all other levels of g, the suit was pressurized by the amount indicated above (1.18 pounds per g).

To obtain some notion of the effect of inspiration on the factors being studied

the first 3 subjects were used to obtain an additional series of 30 roentgenograms taken under the same conditions except that the exposures were made at the end of a 'normal' inspiration.

It should be noted that in the runs at levels of 4 and 5 g without suit pressurization, the centrifuge was stopped as soon as the roentgenogram had been taken. In this way the latent period was rarely exceeded and complete blackout was not produced.

After the roentgenograms had been developed, measurements were made as indicated in figure 1. This illustration was obtained by tracing the pertinent contours on x-ray plates obtained at the end of expiration from one of the subjects exposed to 5 g with and without suit pressurization. The average values for the group of 6 subjects have been substituted for those of this particular subject to provide a more reliable indication of the changes which occurred.

For the measurement of the vertical distance from heart to brain, the point of juncture of the right auricle and aorta (or superior vena cava) on the right border of the cardiac silhouette was selected as a reference point which could be consistently observed. This point is near the level of the aortic valve and in those roentgenograms in which the cardiovascular angle on the left border was clearly discerned, these two points appeared to be on very nearly the same level. The base of the skull could be routinely identified and was chosen as the upper limit of the column of blood extending from heart to brain.

In addition to the information obtained by roentgenograms, respiratory excursions were recorded using a Benedict-Roth B.M.R. apparatus mounted behind the seat on the cab of the centrifuge. Records were obtained during a series of runs on each of the 6 subjects. By determining the base line during control periods before and after exposure to g and the alteration in the base line induced by g, it was possible to obtain information concerning the direction of change in the position of the diaphragm from an independent source.

RESULTS

While the use of a simple fluid-filled manometer is not the most precise method for measuring the intrarectal pressure under the influence of acceleration, it has proved to be a very dramatic method of demonstrating the relatively small influence of large pressures externally applied to the abdomen under these conditions. The linear measurements of the height of the column of fluid in the manometer and the distance from the rectal balloon to the dome of the diaphragm on the right were made as indicated in figure 1. At 1 g at the end of expiration the average manometer pressure was 34 cm. of saline (sp. gr. 1.055) while the distance from the balloon to the diaphragm was 36 cm. The corresponding average values obtained at the end of inspiration were 35 cm. and 32 cm., respectively. Since the pressure registered in the manometer should give some indication of the pressure being exerted on the vessels of the splanchnic circulation, it is evident that the vascular walls are well supported by the intra-abdominal pressure.

Under the influence of radial acceleration the effective weight of the fluid in

the manometer and the abdominal organs increased in direct proportion to the applied acceleration. For this reason the linear measurements of the fluid column in the manometer, and also the height of the column of abdominal organs, were multiplied by the magnitude of the applied acceleration in g's (figure 2).

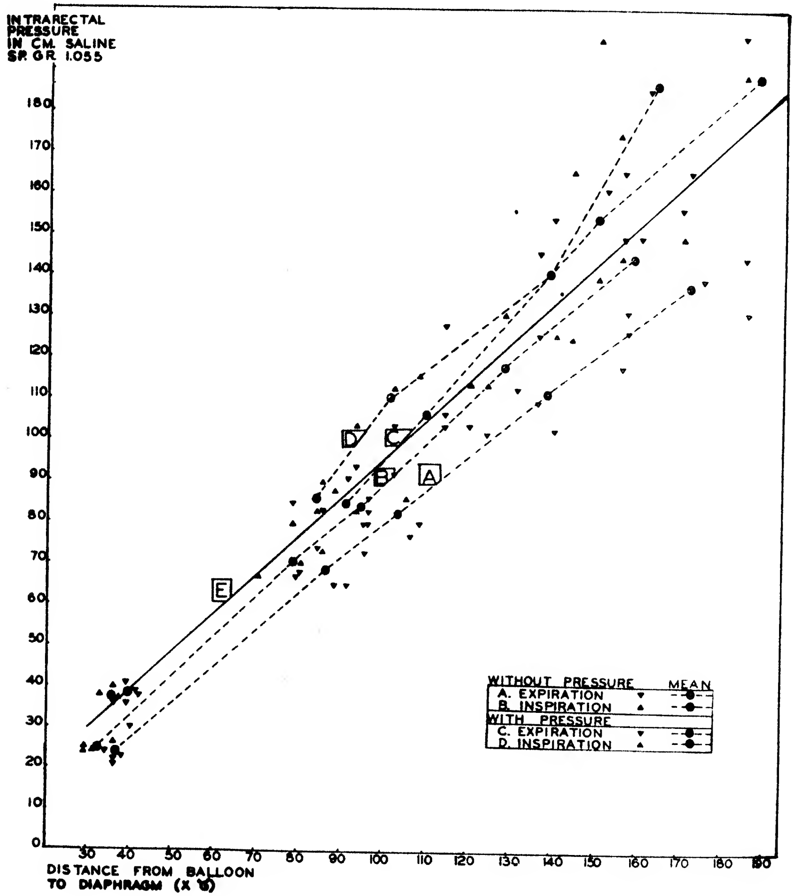

FIG. 2. RELATION OF THE ACTUAL INTRARECTAL PRESSURE to the effective height of the hydrostatic column of abdominal organs is demonstrated by multiplying the linear measurements by the magnitude of the radial acceleration in g. Line E represents a regression coefficient of 1.0.

If the effective specific gravity of the abdominal organs were the same as blood (1.055) and there were no externally applied pressures (e.g., intrathoracic, muscular or from the abdominal bladder), all the points should fall on line E. This statement is based on the concept that under these conditions the abdomen and the manometer would be equivalent to a U tube filled with fluid so that the level

of fluid in the two arms would be equal even during acceleration. Line *A* connects the mean values for the series of determinations obtained without pressure at the end of expiration. Line *B* represents the effect of inspiration in subjects without pressurization. Lines *C* and *D* indicate the results obtained with pressure in the suit at the various levels of g with expiration and inspiration, respectively. With pressurization of the suit most of the values fell above line *E*, indicating that the pressure in the abdomen was sufficient to support a column

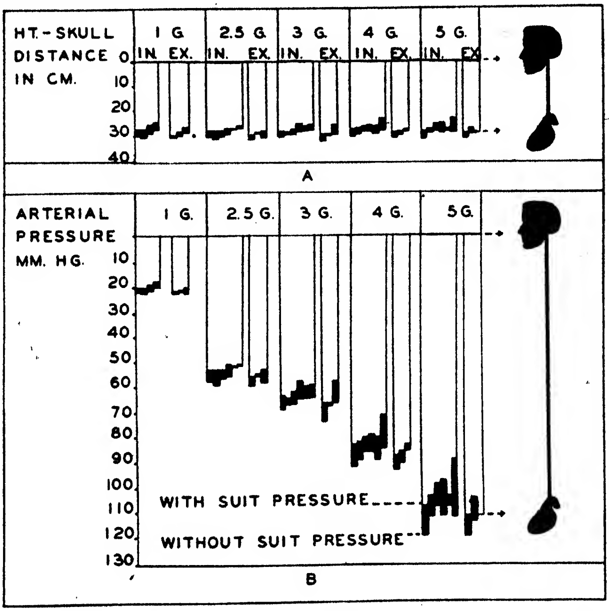

FIG. 3A. LINEAR DISTANCE from the base of the heart to the base of the brain during inspiration and expiration at various levels of acceleration. The black areas represent the reduction in the heart-skull distance resulting from pressurization of the anti-blackout suits.

FIG. 3B. ARTERIAL PRESSURE AT HEART LEVEL required to elevate blood to the base of the skull under the influence of positive g. The black areas represent the protection provided by the anti-blackout suit due to elevation of the heart and reduction in the heart-brain distance.

of blood from any point in the splanchnic reservoir to a level above the diaphragm without a contribution by the vascular walls. The effect of pressurization of the suit was slight considering the fact that the applied pressure amounted to 62 mm. Hg per g or more than 200 mm. Hg at 5 g. It is difficult to assess just how much influence pressurization might have on venous return or blood pressure at heart level.

In accordance with the method illustrated in figure 1, the distance from the junction of the right auricle and ascending aorta (or superior vena cava) to the base of the skull was measured on each roentgenogram. The values obtained

from each subject with and without pressurization of the suit and at the two phases of respiration are diagrammatically presented in figure 3A. As in the case of the hydrostatic pressure in the abdomen, it is necessary to multiply these values by the magnitude of the applied acceleration to arrive at a figure which represents the pressure required to support such a column of fluid. Further, a correction was applied which transposed these values from centimeters of blood to millimeters of mercury. The results of these procedures are presented in figure 3B. These data allow the assignment of specific values for this series of subjects for both the minimum pressure required to elevate blood from the heart to the brain and a measure of the improvement in g tolerance provided by anti-blackout equipment due to reduction in heart-brain distance. At 5 g the reduction in heart-brain distance by means of the anti-blackout equipment produced an average decrease of 11.7 mm. Hg in the pressure at heart level required to support the blood to the base of the skull. Since this represented roughly half the pressure required to support such a column of blood at 1 g, this factor provided about 0.5 g protection. It is interesting to note that the protection provided by the abdominal bladder alone has been reported to be 0.7 g (1).

Spirometer records of the respiratory activity during the experiments revealed that, during acceleration without pressurization of the suit, the base line at expiration shifted from the control base line due to an increase of air within the lungs of about 200 to 600 cc. This finding tended to confirm the observation that the diaphragm assumes a lower position in the chest under these conditions. On the other hand, there was a reduction of similar magnitude in the quantity of the air in the lungs when pressure was applied to the suit. There was marked variability between subjects although repeated runs on the same subjects were fairly consistent. Further studies of this phenomenon have been initiated by other investigators in the laboratory.

DISCUSSION

A review of the stages in the development of anti-blackout equipment has been presented by Wood *et al.* (1). The importance of the rôle of pressure applied over the anterior abdominal wall in improving tolerance to g was universally recognized and led to a series of experiments designed to clarify the nature of the intra-abdominal pressure and its reponse to positive radial acceleration with and without pneumatic anti-blackout suits (2, 3, 4). The intra-abdominal pressure was found to be primarily a hydrostatic type of pressure produced by the column of moveable abdominal organs. Measurements of the intrarectal pressure proved reliable in obtaining information concerning the magnitude and changes in the pressures within the peritoneal cavity. The venous pressure was believed to be partially balanced by the intra-abdominal pressure so that the vein walls should be required to support only a fraction of the venous pressure present at any level in the abdomen. During exposure to positive radial acceleration on the human centrifuge, the intrarectal pressure increased in proportion to the magnitude of the applied force. However, the slope of the curve indicated that the height of the column of abdominal organs was progressively reduced with in-

creasing amounts of centrifugal force. This was attributed to protrusion of the anterior abdominal wall with descent of the diaphragm in the trunk (3, 4). The application of pressure to the anterior abdominal wall appeared to restore the diaphragm to its normal level or above as judged by the response of the recorded intrarectal pressure. Clark and Jorgenson (5) reported an increase in tolerance in relaxed subjects following ingestion of food and fluid which was associated with an increase in the levels of intrarectal pressure during acceleration. Further, they provided confirmatory evidence of the hydrostatic nature of the intrarectal pressure response and noted a marked increase in these pressures associated with voluntary self-protective maneuvers, particularly when anti-blackout suits were used (6).

Lambert and Wood (7), recording pressures from the radial artery supported at the level of the third interspace at the sternum, found that systolic pressure at that level decreased by 4 mm. Hg per g while the diastolic pressure tended to remain unchanged. In subjects wearing pneumatic anti-blackout suits, the systolic and diastolic pressures were higher by 8 and 5 mm. Hg per g, respectively (8). The fall in arterial pressure at head level was much greater, apparently due to the increase in effective weight of the column of blood extending from the base of the heart to the base of the brain.

Thus available evidence indicated that during exposure to positive g, the splanchnic vessels are quite well supported by the intra-abdominal pressure, the diaphragm (and presumably the heart) are depressed within the thorax, the arterial blood pressure at heart level is somewhat reduced and that the most important factor in the reduction in arterial pressure at head level is the height of the column of blood extending from the heart to the brain.

The protection provided the circulation by the peneumatic anti-blackout suits seemed to depend upon a relatively small generalized increase in intra-abdominal pressure (with augmented venous return) and elevation of the diaphragm (and heart) and reduction in the heart-brain distance, plus the increased peripheral resistance and reduced blood content of the lower extremities resulting from pressure applied to the legs.

The present study provides confirmatory evidence for some of the concepts of the mechanism of action of anti-blackout suits, but requires re-evaluation of others. For instance, the data speak eloquently for the concept that the intrarectal pressure reflects a hydrostatic type of pressure resulting from the mass of the abdominal organs. The protection provided by this pressure against pooling within the splanchnic vessels both under normal conditions and during exposure to radial acceleration appears to be remarkably complete. Plotting the intrarectal pressure against the height of the hydrostatic column of abdominal organs (fig. 2) failed to disclose any significant progressive fall in intrarectal pressure below predicted levels with increased amounts of acceleration as previously reported (3, 4). This is in accordance with the concept that this fall had resulted primarily from the descent of the diaphragm, reducing the hydrostatic column of abdominal organs. Contrary to the previously reported opinion (3, 4) that the changes produced by the pressurization of the abdominal bladders

to the extent of one pound per g might be explained solely in terms of elevation of the diaphragm, there appears to be an additional increase in intrarectal pressure associated with stretching of the diaphragm. When an external pressure of 62 mm. Hg (1.18 pounds) per g was applied to the abdomen, the average increase in intrarectal pressure ranged between 5 and 10 mm. Hg per g. Of this, about one third appears to be the result of elevation of the diaphragm.

The venous return from the splanchnic circulation should be significantly increased by the anti-blackout suit on the basis of the results illustrated in figure 2. The limitation on the amount of pressure applied to the abdomen in anti-g suits has been the discomfort produced. The results of this study indicate that this discomfort is most likely to be the result of excessive tension applied to the diaphragm.

At 5 g the use of the anti-blackout suit reduced the average distance from the base of the heart to the base of the skull by 3 cm. This results in a reduction in the blood pressure at heart level required to elevate blood to the brain amounting to about 11 mm. Hg and is roughly equivalent to 0.5 g protection from this source alone. Wood and Lambert (8) reported an increase in arterial blood pressure at the third interspace of 5 to 8 mm. Hg per g from the use of anti-blackout equipment. A significant fraction ($\frac{1}{2}$ to $\frac{1}{4}$) of this improvement appears to be due to a shift in the position of the heart to a higher level in the chest, which could not be detected using a fixed external reference point.

It should be noted that in this series of experiments the subjects wore well-fitted anti-blackout suits on all runs. For this reason, it constitutes a comparison of conditions with and without pressurization of the pneumatic bladders. The suit itself may prevent, to some extent, anterior displacement of the anterior abdominal wall. For this reason the changes observed may not be as great as might be obtained if the effects of pressurization of anti-blackout suits were compared with those produced without any abdominal support.

SUMMARY

1. Roentgenograms were obtained of the head and trunk of subjects exposed to positive radial acceleration with and without pressurization of peneumatic anti-blackout suits. In this way measurements of the changes in the height of the hydrostatic column of abdominal organs, in the intrarectal pressure and in the distance from heart to brain have been obtained.

2. The diaphragm is depressed during exposure to radial acceleration and elevated above its normal level by pressurization of the abdominal bladder with a pressure of 1.2 pounds per g.

3. In addition to the changes in position of the diaphragm, pressurization of the anti-blackout suit increased the overall intrarectal pressure by an amount sufficient to support a column of blood from any point in the abdomen to a level above the diaphragm without a contribution by the vascular walls.

4. The overall increase in intrarectal pressure appeared to be produced by increased tension or stretching of the diaphragm.

5. The distance from the base of the heart to the base of the skull was reduced

by an amount sufficient to provide a protection of about 0.5 g during exposure to 5 g.

6. In addition to this mechanism for protection there is probably an increase in blood pressure at heart level to account for the remainder of the protection produced by the anti-blackout equipment.

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EFFECTS OF A NARCOTIC LEVEL OF CARBON DIOXIDE ON THE PLASMA POTASSIUM AND RESPIRATION OF CATS[1]

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Received for publication August 22, 1947

It is well known that asphyxia causes an increase in the concentration of plasma potassium (5, 8, 10, 13). Mullin, Dennis and Calvin (13) have shown that this is due to anoxia rather than to high CO_2 but Cattell and Civin (4) have reported some preliminary experiments showing that high CO_2 (10%) may also mobilize potassium. The experiments reported here were undertaken to extend this finding by testing the effect of 30 or 40% CO_2 in O_2. The general effect of such mixtures has been studied by Seevers (15). In brief it may be stated that the initial effect on circulation and respiration is dramatic but the animals become rapidly adapted to the lowered pH and live in apparently good condition for many hours. In previously unanesthetized animals such high CO_2 mixtures have a narcotizing effect and can even be used successfully for surgical anesthesia.

METHODS

Normal cats. Full-grown, healthy cats were anesthetized by intraperitoneal injection of 0.75 ml/kgm. of dial. A cannula was introduced into the trachea, and both of the carotid arteries were exposed for the withdrawal of blood samples. The animals was attached by the tracheal cannula to a respiratory recording apparatus. A gas mixture of approximately 35% CO_2 in oxygen was made up for each experiment. The actual CO_2 values ranged from 32 to 38% CO_2, the average for all experiments being 34%. This CO_2-O_2 mixture was stored in a large spirometer from which it passed through a one-way water valve[2] as the cat inspired. The cat could be switched easily to breathing room air by means of a T-tube and a clamp in the inspiratory system. The expired air passed through a second water valve and was collected in a smaller spirometer equipped with a scale which could be read clearly at frequent intervals. A tambour inserted into the expiratory system recorded, by means of pressure differences, the frequency of respiration on a slowly revolving kymograph. Readings of the expired air spirometer were made every one to 3 minutes, except in the period immediately following the change to high CO_2 when the readings were taken every $\frac{1}{4}$ to $\frac{1}{2}$ minute. Before switching the animal to the CO_2-O_2 mixture, a normal period of respiration was obtained and a normal blood sample was taken.

[1] This work was done in part under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of Rochester.

[2] A tube dipping 1 cm. under water through which the air bubbles.

Potassium was determined in duplicate on one ml. of plasma obtained by centrifuging 3 ml. of heparinized blood drawn from the carotid artery. The method used was that of Shohl and Bennett (16), as modified by Fenn and Cobb (7). A few further modifications in technique have been developed over a period of years by various members of this department.

Care was taken to limit the amount of blood withdrawn to 1.5% of the body weight in order to avoid a rise in potassium due to hemorrhage.

Adrenalectomized cats. Following the operative procedure for normal cats, adrenalectomy was performed. The incisions were made below the level of the last rib on each side and the adrenal was removed in one piece, after the blood supply was ligated and cut. Care was taken to keep blood loss at a minimum.

A control experiment was done with an adrenalectomized cat on the respiratory apparatus, but without the administration of CO_2. The potassium level was followed for 5½ hours. Throughout the entire experiment the potassium level varied only within the limits of 1 mEq/l. This is good evidence that the operative procedure caused no large changes in the potassium level over a considerable period of time.

Evisceration technique. After completion of the routine tracheotomy and exposure of the carotid arteries, a midline abdominal incision was made from the xiphoid process of the sternum to the lower abdomen. The intestines were gently lifted out and wrapped in gauze wet with warm saline. As rapidly as possible the following blood vessels were doubly ligated and cut in the same order as they are listed: inferior mesenteric artery, superior mesenteric artery, coeliac artery, portal vein. The incision was then closed and the experiment with the administration of high CO_2 was begun. These animals were, for all practical purposes, without intestines, stomach, spleen, pancreas and liver.

Two operative control experiments showed that the plasma potassium level rose about 1 to 1.5 mEq/l. from the preoperative normal sample to the level observed an hour after the operation. As a general rule, these animals died in about an hour after the operation and the beginning of the high CO_2, in contrast to the normal and adrenalectomized animals which lived for several hours.

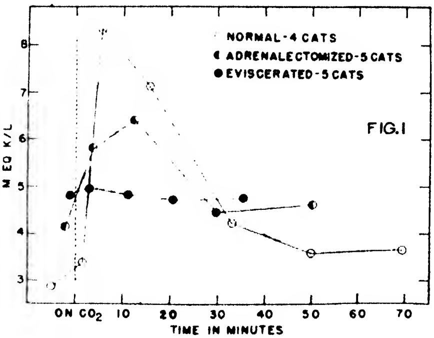
RESULTS

Potassium values. The average potassium curves for normal, adrenalectomized and eviscerated cats are plotted together in figures 1 and 2. The data are arranged to show the potassium changes directly after beginning the administration of 34% CO_2 (fig. 1) and the changes occurring when the CO_2 is removed (fig. 2). The potassium values for each cat, showing the scatter of values averaged to give the points on the graphs, are presented in tables 1 and 2. The potassium values were grouped arbitrarily for averaging according to the time of the samples. Both the potassium values and the times were averaged to give the points on the three curves. It can be seen that the normal cats respond to the high CO_2 with an average increase in plasma potassium from 2.89 to 8.32 mEq/l. This peak is reached in 5.5 minutes from the beginning of the CO_2.

That the dial anesthesia does not cause a marked change in blood potassium in cats over a period of 4 to 8 hours has been shown by Cattell and Civin (4).

The mechanism by which the potassium is liberated into the blood has been thought to be due to: *a*) the effect of CO_2 in stimulating the adrenals which release adrenaline; adrenaline then affects the liver to release potassium; and *b*) the direct effect of CO_2 on the liver to release potassium.

In an attempt to determine whether or not the curves for normal and adrenalectomized cats were significantly different, the increments from the normal of all potassium values within the first 30 minutes of CO_2 were recorded for both groups. These data were then treated statistically and by the critical ratio test were shown to have a C.R. of less than 2. By Fisher's *t*-test, the probability was $0.2 > P > 0.1$. This indicates that the data are not adequate



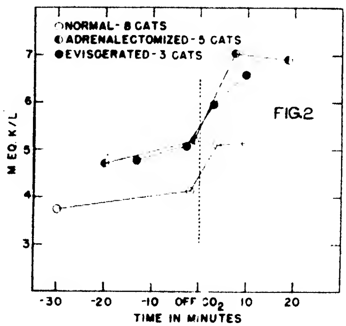


FIG. 1. CHANGE IN PLASMA POTASSIUM in normal, adrenalectomized and eviscerated cats breathing 32 to 38% CO_2 in oxygen.

FIG. 2. CHANGES IN PLASMA POTASSIUM in normal, adrenalectomized and eviscerated cats on the return to breathing air after high CO_2.

to prove a significant difference between the potassium responses of normal and adrenalectomized cats.

Following the rapid rise of plasma potassium in the normal cats, there was a steady, less rapid decline approaching the pre-CO_2 normal value in about 30 minutes. In both cases the level maintained after the decline was 0.5 to 0.7 mEq/l. greater than the original potassium value.

It will be noted that the normal potassium values before the administration of CO_2 were considerably higher for the adrenalectomized group than for the normal cats. This was presumably due either to the trauma of the operation or more specifically to absence of the adrenals.

In contrast to the normal and adrenalectomized cats, the groups of animals which were eviscerated prior to the administration of high CO_2 did not show the characteristic rise in plasma potassium. The average values for all cats show almost a straight line, the range of variation being within 0.25 mEq/l. All of the experimental points making up the curve fell between 4.0 and 6.0 mEq/l.,

a very narrow range compared to the other two experimental groups. As in the case of the adrenalectomized animals, the normal pre-CO_2 potassium value is elevated in the eviscerated group. This is thought to be caused by the stimulation and trauma of the operative procedure (17) although every effort was made to keep this at a minimum.

TABLE 1. *Plasma potassium changes in cats breathing high CO_2*

Adrenalectomized

CAT NO. 1—3.3 KGM.		CAT NO. 2—4.2 KGM.		CAT NO. 3—2.0 KGM.		CAT NO. 4—4.3 KGM.		CAT NO. 5—3.3 KGM.	
Time[1]	K[2]	Time	K	Time	K	Time	K	Time	K
−3.0	3.75	−1.0	4.56	−2.0	2.62	−2.5	5.46	−2.0	4.25
3.0	3.75	5.5	8.83	2.0	2.69	3.0	6.56	3.5	7.18
10.0	5.94	18.0	5.43	13.0	4.38	10.5	9.62	10.0	6.63
31.0	3.63	35.0	3.50	28.0	3.50	32.0	6.37	22.5	4.75
51.0	3.63	55.0	4.80	42.0	3.32	52.0	4.94	51.5	6.31

Eviscerated

CAT NO. 6—3.1 KGM.		CAT NO. 7—2.8 KGM.		CAT NO. 8—2.4 KGM.		CAT NO. 9—2.1 KGM.		CAT NO. 10—3.6 KGM.	
Time	K	Time	K	Time	K	Time	K	Time	K
−1.5	5.50	−1.0	3.62	−0.5	4.75	−1.0	4.75	−2.0	5.37
3.0	4.50	3.0	4.62	3.5	6.00	2.0	4.50	3.0	(5.08)
8.0	4.87	10.0	5.37	16.0	5.12	8.0	4.25	14.0	4.50
20.0	(4.87)	20.0	4.56	22.0	5.25	16.0	4.75	24.0	4.00
35.0	(4.87)	34.0	4.75	35.0	(5.25)	27.0	4.75	46.0	4.12

Normal

CAT NO. 11—2.9 KGM.		CAT NO. 12—2.3 KGM.		CAT NO. 13—1.7 KGM.		CAT NO. 14—2.3 KGM.	
Time	K	Time	K	Time	K	Time	K
−5.0	1.72	−2.5	2.72	−7.0	3.38	−6.0	3.75
2.0	2.44	0.5	3.34	1.0	3.08	2.5	4.67
9.0	10.95	3.5	9.00	3.0	6.21	6.0	7.13
14.0	10.00	15.0	(6.80)	19.5	4.88	13.0	6.75
36.0	5.12	32.0	3.62	32.0	(4.10)	32.0	4.00
49.0	3.56	50.0	(3.53)	45.5	3.37	55.0	3.87
71.0	3.00	63.0	3.50	70.0	(3.83)	73.5	4.19

Values in parentheses were interpolated.
[1] Time measured in minutes before (minus) and after (plus) onset of CO_2 breathing.
[2] Potassium values are expressed in mEq/l.

Figure 2 presents the average potassium values for the three groups of animals as the CO_2 was removed. All three curves show an immediate rise in potassium concentration of 1 to 2 mEq/l. Some differences might have appeared in a more extended recovery period.

Respiration values. The effect of 34% carbon dioxide in oxygen on the res-

piration of normal, adrenalectomized and eviscerated cats is presented in figure 3. The three upper graphs show the changes in tidal volume while the lower three express the changes in minute volume. The normal, highest and lowest tidal volumes for all cats were recorded and averaged at suitable intervals.

TABLE 2. *Plasma potassium changes in cats on removal of CO_2*

Adrenalectomized

CAT NO. 1—3.3 KGM.		CAT NO. 2—4.2 KGM.		CAT NO. 3—2.0 KGM.		CAT NO. 4—4.3 KGM.		CAT NO. 5—3.3 KGM.	
Time[1]	K[2]	Time	K	Time	K	Time	K	Time	K
−25.0	4.38	−23.0	3.50	−15.0	3.50	−14.0	5.86	−23.0	6.31
−1.0	5.00	−1.0	4.80	−1.0	3.32	−2.0	6.75	−1.0	6.12
9.0	5.69	8.0	6.56	8.0	5.13	6.0	10.00	6.0	7.86
17.0	5.79	17.0	(6.56)	25.0	4.32	18.0	10.75	16.0	7.75

Eviscerated

CAT NO. 6 —3.1 KGM.		CAT NO. 8—2.4 KGM.		CAT NO. 9—2.1 KGM.	
Time	K	Time	K	Time	K
−23.0	4.50	−7.0	5.12	−10.0	4.75
−6.0	4.87	−1.0	5.25	−1.0	5.12
3.0	5.50	4.0	5.76	2.0	6.62
10.0	6.61	9.0	6.50	10.0	(6.62)

Normal

CAT NO. 11—2.9 KGM.		CAT NO. 12 —2.3 KGM.		CAT NO. 13—1.7 KGM.		CAT NO. 14—2.3 KGM.	
Time	K	Time	K	Time	K	Time	K
−31.0	3.00	−34.0	3.62	−43.0	3.37	−20.0	3.87
−4.0	3.06	−3.0	3.50	−2.0	4.12	−1.5	4.19
3.0	3.38	1.5	3.56	1.5	5.37	5.0	5.68
6.0	3.56	9.0	3.41	5.5	5.41	14.0	6.68
CAT NO. 15—2.8 KGM.		CAT NO. 16—1.7 KGM.		CAT NO. 17—2.4 KGM.		CAT NO. 18—1.6 KGM.	
Time	K	Time	K	Time	K	Time	K
−13.5	2.63	−41.0	4.87	−31.5	4.56	−24.5	4.00
−1.5	3.31	−0.5	5.56	−0.5	4.93	−2.5	4.43
3.5	4.75	4.5	5.50	5.5	6.63	4.0	6.01
12.5	4.63	10.5	5.25	10.0	(6.78)	10.0	(5.35)

Values in parentheses were interpolated.
[1] Time measured in minutes before (minus) and after (plus) end of CO_2 breathing.
[2] Potassium values are expressed in mEq./l.

In normal cats, the tidal volume and minute volume curves showing the respiratory changes under high CO_2 are remarkably similar from one animal to another. During the first two minutes on CO_2 the average tidal volume increases

145%, while the average minute volume increases about 170%. This respiratory stimulation is followed by a depression after 1.5–2.0 minutes, with both tidal and minute volume falling considerably below the normal values.

After this depression, the tidal volume and minute volume increase and approach the same high values reached during the height of the initial stimulation. The principal difference between the shapes of the curves is that the tidal volume increases more rapidly during the first 20 minutes and begins to level off, while the minute volume maintains a steady increase. This indicates that the frequency of respiration must have increased slightly over the period from 40 to 80 minutes.

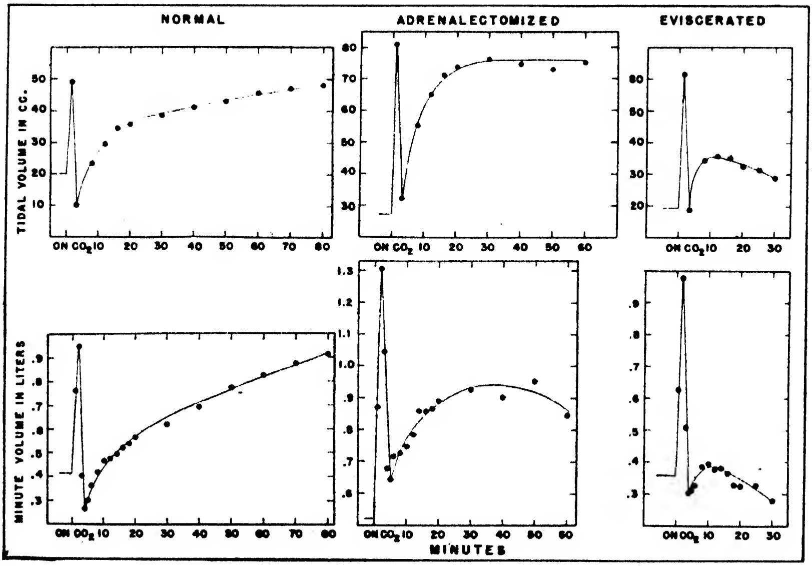


FIG. 3. AVERAGE CURVES showing the effect of breathing 34% CO_2 beginning at zero time. The 3 upper curves represent tidal volumes and the 3 lower curves minute volumes of the respiration in normal, adrenalectomized and eviscerated cats as indicated.

The adrenalectomized cats responded to high CO_2 with almost a 200% increase in tidal volume and 150% increase in minute volume in one to 2 minutes. The subsequent depression differs from that of the normal cats in that the values do not fall below the normal tidal and minute volumes. The increase in tidal volume following the depression rapidly approaches the peak value and levels off a little below this point. The minute volume follows approximately the same course but the rise after the depression is much less, indicating that the frequency decreased in the period from 20 to 60 minutes. The minute volume curve also indicates that the gradual adaptation to the high CO_2 is not the same in the adrenalectomized cats as in the normal cats, since the curve does not show as great an increase in minute volume nor does it continue to rise.

The respiratory response of the eviscerated cats was similar to that already described with two important exceptions: *a*) the respiration record extends for only 30 minutes on high CO_2 since the survival period of these animals was short; and *b*) the recovery after the depression is much diminished.

DISCUSSION

The rise in potassium caused by asphyxia has been attributed to the liberation of adrenaline, since this effect is produced by the injection of adrenaline (1, 2, 5, 6, 12, 14). It has been shown by Houssay, Marenzi and Gerschman (10) that the effect is much diminished when the liver is not available as a source of potassium. That this is not the only mechanism is shown by two experiments of Cattell and Civin (4) in which the rise in potassium was found to be not significantly altered by adrenalectomy. This suggests that the adrenal hepatic mechanism should also be considered as an explanation for the effects of high CO_2. Our experiments with adrenalectomized cats which were designed to test this point certainly show that a rise of potassium is obtained with CO_2 in the absence of the adrenals. While this effect seems to be somewhat smaller in the adrenalectomized animals the variation is such that this result might have occurred by chance in one out of 10 trials. It must be concluded, therefore, that more animals are required to prove that the adrenal-hepatic mechanism is stimulated by the high CO_2, although this effect is not improbable. The rise of plasma potassium caused by CO_2 is less rapid and more prolonged than that caused by the injection of adrenaline, but this may indicate merely a different type of stimulation of the same mechanism.

The fall in potassium which occurs after the initial rise indicates that the CO_2 does more than cause a shift in a physico-chemical equilibrium between cells and tissues. Otherwise the potassium level would remain high until the CO_2 also diminished. Apparently the high CO_2 causes a sudden liberation of potassium into the blood stream, which is then disposed of in the muscles and other tissues in the same way as potassium injected intravenously.

That tissues other than the liver take up some of the liberated potassium in its disappearance from the blood is shown by the series of 4 cats in which the blood supply of the liver was not tied off until after the potassium had been liberated into the blood by CO_2 stimulation. In spite of the absence of the liver the potassium level in the plasma continued to fall. Although the experiments were not conclusive, this finding is in agreement with Houssay and Marenzi (11), who report that the rate of disappearance of injected potassium is not affected by the absence of the alimentary canal, liver or kidney, indicating that the muscles are chiefly responsible for the uptake of potassium.

The work of Fenn (9) has shown that when glycogen is laid down in the liver, it takes with it both water and potassium. When glycogen is liberated therefore as glucose there should be also an increase in the blood potassium. Some unpublished data of Fenn, Adler and Enns tend to support this. A mixture of 35% CO_2 in oxygen was administered to a cat and analyses of potassium, glucose, pH and lactic acid were made. After 10 minutes of high CO_2 the glucose was

found to be 157% and the potassium 137% above the pre-CO_2 level. The glucose did not drop as rapidly as the potassium but appeared to stay at a high level until the CO_2 was discontinued. The pH of the blood as drawn (without loss of CO_2) fell progressively to 6.74 during the time on CO_2 and returned to a pH above normal when air was again breathed. The pH of the same samples equilibrated at 5% CO_2 showed a smaller decrease, probably due to the production of lactic acid, which was observed to increase progressively. These facts suggest that the high CO_2 may stimulate the liberation of glucose from the liver glycogen stores and that this is incidentally accompanied by potassium. More experiments, however, are necessary before such a conclusion could be drawn.

The curves showing the average potassium changes following the removal of the high CO_2 must now be considered. Since the rise in potassium was a small one and because it occurs in normal, adrenalectomized and eviscerated animals in approximately the same amount, it seems probable that it is a result of an acid-base change. The similarity of the curves for the three groups of animals suggests that the potassium must come chiefly from the muscles.

Working with isolated frog sartorius muscles immersed in blood or other solutions, Fenn and Cobb (7) have shown that, in the presence of increased CO_2 tension, potassium shifts from a medium of frog's blood to the muscle. When the muscles were immersed in Ringer's solution and a high CO_2 tension was applied, the potassium moved from the muscle to the Ringer's solution. This evidence suggests that under high CO_2 potassium tends to move from the better buffered medium to the poorer buffered medium. As shown by the CO_2 dissociation curves the muscles were intermediate in their buffering capacity between the blood and the Ringer's solution. Applying these principles to the present experiments, one would expect to find a fall in the plasma potassium on the administration of high CO_2 to cats and an increase when the CO_2 was removed. The latter effect was actually seen (fig. 2) but the initial fall was not seen, probably because, if it were present, it would have been masked by the rise due to the liberation of potassium from the liver. In the eviscerated cats, however, where potassium did not increase, there was no fall of potassium when CO_2 was applied. In support of this idea, on the other hand, is the report of Cattell and Civin (4) that during the first minute of asphyxia in cats, the serum potassium consistently falls, the average amount being 2 mgm. per 100 ml. of serum. This occurs just before the characteristic potassium rise. It may be that the more gradual accumulation of CO_2 gives time for the fall to be seen before it is masked by the rise in potassium due to the liver. The small rise in potassium on the removal of CO_2 can then be explained as the reverse process, a movement of potassium from the muscles to the plasma with the decrease in CO_2 tension.

The similarity in the shape of the tidal and minute volume curves indicates that there is little change in frequency of breathing. As a general rule in all three groups of cats, the frequency diminished slightly at the peak of the stimulation and increased slightly at the depth of the depression. During the period of recovery and adaptation, the frequency decreased again to a value slightly less than that at the height of stimulation.

The initial stimulation of the respiration gives way to depression as the CO_2 passes from the excitatory to the narcotic level. The principal differences seen in the normal, adrenalectomized and eviscerated curves are in the recovery following the immediate stimulation and depression. The slow increase in respiration after the depression, gradually approaching the peak value, appears to reflect some physiological adaptation to the high CO_2. In the adrenalectomized animals the minute volume shows less of an increase following the depression and in the eviscerated animals, still less. This lack of 'adaptation' to the high CO_2 may be connected with the short survival of the operated animals, especially in the latter group.

SUMMARY

1. Normal anesthetized cats breathing an average of 34% CO_2 in oxygen showed a rapid rise in plasma potassium, reaching a peak in 5½ minutes and then gradually returning to near normal in about 30 minutes, while the CO_2 was being continued.

2. Adrenalectomized cats breathing high CO_2 showed a similar but smaller rise. This difference in the size of the response may or may not be significant. This suggests but does not prove the participation of the adrenal-hepatic mechanism in the CO_2 effect but indicates definitely that some other mechanism must also be involved.

3. Cats which were eviscerated prior to the administration of CO_2 showed no rise in plasma potassium when given an average of 34% CO_2 in oxygen.

4. On the removal of CO_2, normal, adrenalectomized and eviscerated cats show a small rise in plasma potassium, probably released from the muscles to maintain the acid-base balance.

5. In all the cats the high CO_2 caused an initial stimulation of respiration, followed by a sudden depression as the CO_2 passed from the excitatory to the narcotic level. The height of the stimulation and the depth of the depression usually occurred within about 5 minutes on CO_2. Thereafter the respiration gradually increased, approaching the stimulation peak value in the case of the normal cats. Neither the adrenalectomized nor the eviscerated cats showed such a remarkable increase in the respiration, as measured by minute volume after the depression, though the adrenalectomized cats did show a very large increase in tidal volume.

The author wishes to express sincere appreciation to Dr. W. O. Fenn for his untiring interest, patient guidance and helpful criticisms throughout this work.

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EFFECTS ON MAN OF HIGH CONCENTRATIONS OF CARBON DIOXIDE IN RELATION TO VARIOUS OXYGEN PRESSURES DURING EXPOSURES AS LONG AS 72 HOURS[1]

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Received for publication August 18, 1947

The limits of 3% carbon dioxide and 17% oxygen in ambient air have been accepted in the American Submarine Service as compatible with efficient performance of personnel for extended periods of time. Although considerable experimental data have accumulated on the influence of carbon dioxide on respiration (1–4), the investigations, with the exception of those of Miller (5), have been limited to short-term exposures. Furthermore, other than the studies of Case and Haldane (6), little attempt has been made to correlate the changes in respiration caused by combined oxygen deficiency and carbon dioxide excess with psycho-physiological functions.

With respect to the oxygen saturation of blood, the prime consideration is not the partial pressure of oxygen in ambient air, but the much lower alveolar oxygen pressure. If the lungs could be more effectively ventilated, it should be possible to raise the alveolar oxygen pressure to levels approaching that in the ambient air. The problem is to determine the carbon dioxide concentration in the ambient air that will bring about maximal pulmonary ventilation without undue physical impairment. This will permit the alveolar oxygen pressure to approximate the partial pressure of oxygen in ambient air.

TEST PROCEDURES

General

In six experiments of 35 to 72 hours' duration, groups of 4 to 77 male subjects (age range 18 to 45 years) occupied sealed steel chambers which allowed a free air space of approximately 500 cu. ft. per man. The first experiment was an indoctrination run. In the second experiment of 52 hours' duration (4 subjects), the exhaled carbon dioxide was not absorbed and oxygen was not replenished. In the third experiment (4 subjects), carbon dioxide likewise was not absorbed but the ambient oxygen was not permitted to fall below 19%. In the fourth experiment of 72 hours' duration (4 subjects), carbon dioxide in excess of 5% was absorbed; oxygen was not replenished. In experiments 5 and 6, the carbon dioxide in excess of 5% was again absorbed; oxygen was not replenished. In experiment 5, 37 men breathed recirculated air for 60 hours and in experiment 6, 77 men were subjected to similar conditions for 50 hours.

[1] The material in this article should be construed only as the personal opinions of the writers and not as representing the opinion of the Navy Department officially.

In the first four experiments an Effective Temperature of approximately 85° was maintained to simulate hot tropical conditions with a dry bulb of 90° F. and a relative humidity of 75%. In experiment 5, the Effective Temperature averaged 75° with a dry bulb of 80° F. and a relative humidity of 65%; in experiment 6, the Effective Temperature averaged 59°, with a dry bulb of 60° F. and a relative humidity of 90%.

Biochemical, physiological and psychological measurements and observations were made. The following daily schedule was followed in the first four experiments and slight modifications were made in experiments 5 and 6.

0800–1030	psychological tests	1400–1800	test program repeated
1030–1130	physiological tests	1800–2000	dinner—rest period
1130–1200	biochemical tests	2000–2400	test program repeated
1200–1400	lunch—rest period	2400–0800	sleep period—breakfast

Biochemical

In the early experiments, blood was drawn from the brachial artery. Due to the frequency of needle insertion as well as technical difficulties, samples of 'arterialized' venous blood were drawn in the later experiments. These were obtained by immersing the hand in hot water (45°C.) for 20 minutes and with the hand still immersed, drawing blood from one of the dorsal veins of the hand (7). Blood obtained in this manner was used for gas analysis in lieu of arterial blood.

Alveolar air samples were taken according to the technic described by Dill (8). All subjects were trained for several days before the start of the experiments to insure proper sampling technic.

The plasma pH was calculated by means of the Henderson-Hasselbalch equation from data obtained from analysis of alveolar air and arterial blood or 'arterialized' venous blood (9, 10).

Physiological

The following measurements were made in the course of the experiments: pulse rate, blood pressure, body temperature, pulse rate response to exercise and respiratory rate and minute volume. The observers followed a strict routine in making all measurements in order to reduce to a minimum the variability in data usually obtained with inexperienced subjects. During a typical test procedure, the subject reclined quietly for 15 minutes, after which the pulse rate, blood pressure and body temperature were obtained. He was next allowed to assume a sitting position while the respiratory measurements were made. Finally, he engaged in light activity for the purpose of recording response to exercise.

Pulse beats were counted for 30 seconds. Blood pressure was measured by auscultation, the diastolic pressure being taken at the point of sound disappearance. Pulse pressure was computed as the difference between systolic and diastolic pressures. Body temperature was obtained with standard clinical thermometers, rectal temperatures being employed in the first four experiments,

and oral temperatures in the last two. To obtain the respiratory data in the first four experiments, expired air was collected by means of a face mask connected to a Tissot spirometer; dry gas meters were employed in experiments 5 and 6 in place of spirometers. The respiratory rate was counted for a full minute and minute volume was measured for a period of 5 minutes. Exercise response was evaluated on the basis of performance in the step-up test (11). For this test the subject stepped up and down, using the same leg, on an 18-inch box, 20 times in 30 seconds, pulse counts being made immediately after and two minutes after cessation of exercise. A 'cardiovascular score' was computed by the formula (11):

C.V.S. = (5″ to 20″ pulse count) plus (1′45″ to 2′15″ pulse count).

Psychological

Fourteen different tests were used in the psychological battery. Principles which guided the selection of tests were: that test procedures cover a wide range of functions; that tests have high enough reliability to make possible an evaluation of individual performance; and that tests be used that were known to be satisfactory as criteria of anoxia. The functions tested were:

Vision. Foveal flicker frequency was measured as one significant aspect of central photopic vision (12). The dark-adapted form-acuity threshold was measured with a T-shaped test object similar to that of the Navy radium plaque adaptometer (13).

Audition. Measures were made of the ability to discriminate differences in pitch and loudness (14), and of the absolute auditory threshold over a wide range of pitch.

Equilibrium. Ability to stand still and erect was measured by recording anterior-posterior body sway with eyes open and closed (15). Ability to maintain balance during movement was measured by requiring the subject to walk a one-inch rail without shoes (15).

Hand-arm steadiness. This was measured by the ability to keep the end of a rod in a fixed position.

Eye-hand coordination. Two tests of this function were used: the Koerth pursuit rotor, which requires a smooth continuous pattern of movement for one hand; and a complex tapping test, which requires irregular and non-symmetrical movement of both hands simultaneously (16).

Strength. A Smedley hand dynamometer was used, following a procedure that requires steadily increasing outputs of energy to the point where the subject is no longer able to improve (17).

Symbolic functions. Three paper-and-pencil tests were used: the Johnson Code Test, which requires continuous application and attention in a series of letter-for-letter translations (18); the computation test, which is a series of mixed addition and subtraction problems (18); and the number-checking test, which requires the comparison of pairs of numbers to determine whether they are alike or different (19).

Except in experiment 6 and on one test in experiment 5, all subjects had ex-

tended practice on the tests before the experiments began, in order to minimize the effect of rapid learning and irregular adaptation to test conditions. Control of motivation was not possible, but there was reason to believe that motivation was relatively high and constant. The subjects knew the purpose and nature of the research and knew approximately, if not exactly, how well they were doing on each test. There were no special rewards or inducements to good performance but a general social facilitation and normal competitive spirit developed among the subjects; i.e., morale was judged to be good.

EXPERIMENTAL DATA AND DISCUSSION

General

The cost of maintaining adequate oxygenation of hemoglobin when the ambient oxygen is as low as 12% and carbon dioxide as high as 5% is an approximate 2½-fold increase in minute breathing volume, a rise in pulse rate of approximately 10 beats per minute, some impairment in specific sensorimotor performance,

TABLE 1. *Summary of conditions to which the subjects were exposed*

EXPERIMENT NUMBER	NUMBER OF MEN	DURATION	HIGHEST CO_2	LOWEST O_2	HOUR WHEN AMBIENT CO_2 APPROACHED 5%
		hrs.	*per cent*	*per cent*	
1	4	34	5.95	14.18	29
2	4	52	6.54	13.45	34
3	4	51	6.75	19.22	37
4	4	72	5.42	10.45	32
5	37	60	5.27	12.21	34
6	77	50	5.18	13.21	34

headache affecting 20% of the personnel, and occasionally nausea. Throughout all experiments involving 130 man exposures, only three men were removed from the closed spaces. One of these men showed apprehension; another, exhaustion; and a third, a steadily increasing blood pressure. At no time were these men in a critical condition.

The period immediately following inhalation of outside air may be attended by transient dizziness and headache. Observers exposed periodically for one to two hours to the recirculated compartment air repeatedly developed headaches and experienced a transient taste and smell of ammonia upon leaving the compartment. However, the apparently complete recovery of both subjects and observers was rapid.

The data (table 1) show that the highest carbon dioxide concentration was 6.75%, the lowest oxygen concentration 10.45%. Although these concentrations were tolerated, the symptoms of headache and respiratory difficulty, especially during physical effort, sharply increased whenever the carbon dioxide rose appreciably above 5%.

Special significance is attached to experiment 3 in which the oxygen was main-

tained at a level approaching normal although the carbon dioxide was permitted to approach 7%. In this experiment it was found that measurable improvement over the performance in other experiments did not result from the added oxygen.

The remarkably consistent values of carbon dioxide output per man, of 0.326 l/min. STP (0.69 cu. ft/hour) and oxygen consumption 0.387 l/min. STP (0.82 cu. ft/hour), agree with those obtained by previous investigators in tests performed during and subsequent to the first World War.

It is noteworthy that the alveolar carbon dioxide pressure, respiratory minute volume, and pulse rate show very little change until the atmospheric carbon dioxide approaches the 3% level. Above the 3% level, these functions begin to increase rather sharply.

Biochemical Data

Ambient carbon dioxide and oxygen concentrations. In experiment 3, the carbon dioxide rose to a value of 6.75 at the end of 51 hours but the oxygen was maintained at a level of 20% ± 0.8 during this period (fig. 1). In experiment 4, carbon dioxide absorption was begun at the end of 45 hours to maintain the carbon dioxide level at 5% until the termination of the test at the end of 72 hours. Oxygen was not added and the concentration fell to 12.8% during the 56th hour where it remained for several suceeding hours (fig. 2). At this time, nitrogen was added at a constant rate to compensate for outboard leakage. In experiments 5 and 6, carbon dioxide absorption was begun during the 35th hour when the concentration reached 5% and continued for 25 and 15 additional hours, respectively (fig. 3, 4).

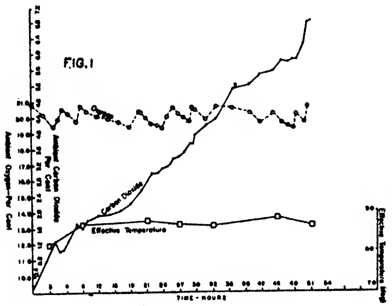
Comparison of values of ambient with alveolar carbon dioxide and oxygen concentrations. As a result of the increased ventilation, the oxygen percentage in the lungs during the rebreathing of air falls at a slower rate than it does in the ambient air. The difference between ambient and alveolar oxygen pressures (ΔpO_2) varies from 40 to 52 mm. Hg at the beginning of the tests and declines steadily to values ranging from 11 to 19 mm. Hg at the conclusion of the experiment (table 2, fig. 5). The difference between alveolar and ambient carbon dioxide (ΔpCO_2) diminishes similarly from about 42 mm. Hg (chamber open to outside air) to about 10 mm. Hg when the ambient carbon dioxide reaches 46 mm. Hg (exp. 2, table 2).

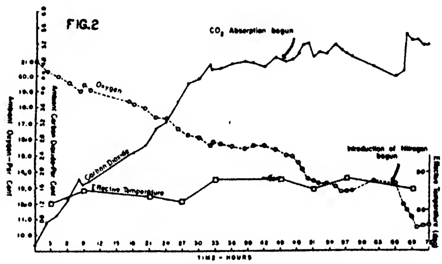
Effects of high carbon dioxide concentrations on plasma pH and carbon dioxide content. The plasma pH values (table 3) indicate a slight increase in acidity, 7.44 to 7.38 in one experiment and 7.40 to 7.38 in another, when the carbon dioxide of the ambient air increased from 0.03% to 5%. Plasma carbon dioxide increased from 58.6 to 59.5 vols. % in one experiment, and from 58.2 to 64.6 vols. % in another. These changes may be classified as slight and are at variance with the results obtained by Miller (5). They further illustrate the remarkable rôle played by hyperventilation in protecting the body against the accumulation of carbon dioxide in the presence of high ambient concentrations of this gas.

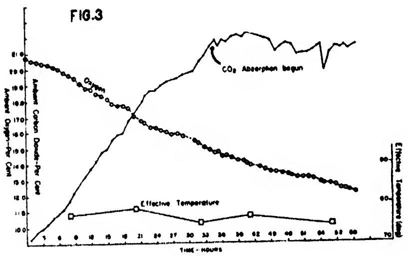
Oxygen concentrations in ambient and alveolar air and the corresponding equivalent altitudes. An ambient oxygen pressure of 100 mm. Hg without increased

carbon dioxide in the air is associated with an alveolar oxygen pressure of 58 mm. Hg. Corresponding values are found at simulated altitudes of 10,000 feet (20). The same ambient oxygen pressure (100 mm. Hg), however, in combination with 5% carbon dioxide in air is associated with an alveolar pressure of 86 mm. Hg. This value corresponds to a simulated altitude of but 4000 feet (fig. 6).

Effects of carbon dioxide on oxygen saturation of blood. The percentages of oxyhemoglobin in two experiments (4 and 5) when carbon dioxide in ambient







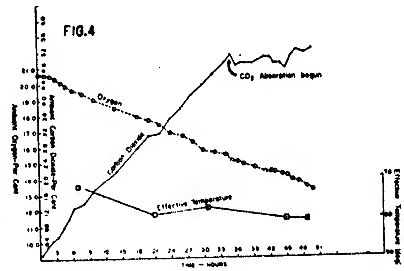


FIG. 1. Experiment 3, the rise in CO_2 maintaining constant O_2 during 52 hours recirculation of compartment air.

FIG. 2. Experiment 4, the rise in CO_2 and fall in O_2 during 72 hours recirculation of compartment air. During the 65th hour nitrogen and some carbon dioxide was introduced into the compartment.

FIG. 3. Experiment 5, the rise in CO_2 and fall in O_2 during 60 hours recirculation of compartment air.

FIG. 4. Experiment 6, the rise in CO_2 and fall in O_2 during 50 hours recirculation of compartment air.

air is 5% show considerable elevation over percentages expected in the absence of CO_2 when the oxygen pressure in inhaled air is reduced (table 3, fig. 7). These relatively high saturation values are due to the maintenance of a high alveolar oxygen pressure (table 2) resulting from hyperventilation. In association with an ambient carbon dioxide pressure of 36 mm. Hg an oxygen pressure of 72.5 mm. Hg (corresponding to that at a simulated altitude of 17,000 feet) saturates hemoglobin 87% (fig. 7). In the absence of carbon dioxide in the inhaled air, the saturation of hemoglobin would have been of the order of 76%. However,

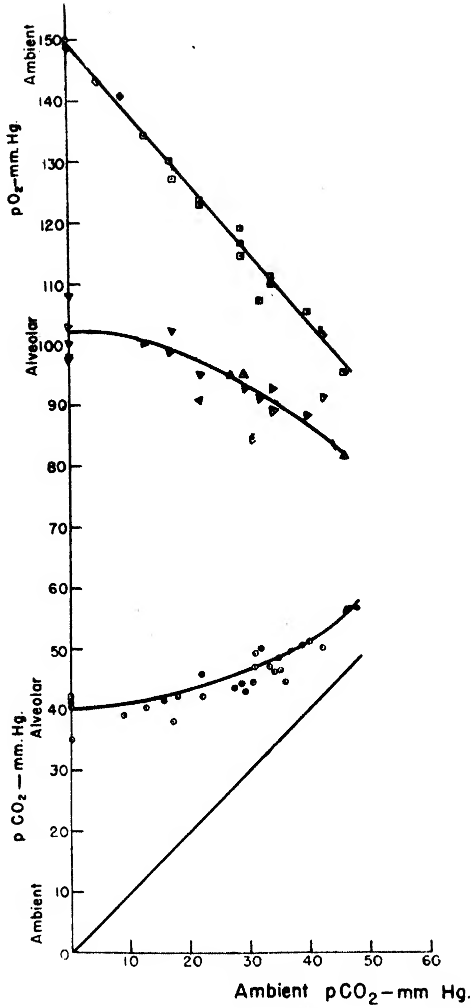
on the basis of the data submitted no further gain in alveolar oxygen or hemoglobin saturation is to be expected by increasing the ambient carbon dioxide

TABLE 2. *Effects of increased ambient carbon dioxide on alveolar air*

NUMBER EXPERIMENT	NUMBER OF SUBJECTS	DATE	HOURS OF EXPOSURE	AMBIENT AIR				ALVEOLAR AIR				ΔpCO_2[1]	ΔpO_2[1]
				CO_2	O_2	pCO_2	pO_2	CO_2	O_2	pCO_2	pO_2		
		1944		*Per cent*		*mm. Hg*		*Per cent*		*mm. Hg*		*mm. Hg*	*mm. Hg*
1	4	11 May	Rest	0.03	20.94	0.2	150.1	5.76	14.18	40.8	100.4	40.6	49.7
	4		4	1.28	19.62	9.2	140.5	5.49	14.60	39.3	103.7	30.1	36.8
	4		10	2.41	18.32	17.3	131.2	5.94	13.75	42.3	98.5	25.0	32.7
	4	12 May	23	3.84	16.63	27.5	119.1	5.81	13.28	43.5	95.0	16.0	22.1
	4		28	4.79	15.50	34.3	111.0	6.50	13.01	46.4	92.5	12.1	18.5
	4		34	5.95	14.18	42.6	101.5	7.08	12.55	50.4	89.3	7.8	12.2
2	4	17 May	Rest	0.03	20.94	0.2	150.1	5.92	14.55	41.9	103.0	41.7	47.0
	4		4	0.75	20.23	5.3	143.4	4.90	16.05	33.7	112.8	26.6	30.6
	4		10	1.79	18.97	12.6	134.4	5.74	14.20	40.4	100.0	27.8	34.4
	4	18 May	22	3.15	17.48	22.3	123.7	5.95	13.49	42.2	95.2	19.9	28.5
	4		28	4.07	16.42	28.8	116.3	6.22	13.09	44.1	92.9	15.3	23.4
	4		34	4.83	15.50	34.1	109.6	6.61	12.95	46.3	89.0	12.2	20.0
	4	19 May	46	5.66	14.52	40.0	102.8	6.93	12.39	49.3	88.2	12.3	18.4
	4		51	6.54	13.45	46.2	95.2	7.87	11.45	55.8	81.4	9.6	13.8
3	4	25 May	Rest	0.03	20.94	0.2	150.1	5.81	13.85	41.1	98.1	39.9	52.0
	4		18	2.21	19.34	15.9	138.5	5.85	14.84	41.4	105.6	25.5	32.9
	4		34	4.32	20.57	31.0	147.5	6.57	17.79	46.8	127.0	15.8	20.5
	4	26 May	42	5.41	19.54	38.8	140.0	7.10	16.86	50.7	123.7	11.9	16.3
	4		51	6.72	20.52	48.2	147.2	7.92	18.98	56.7	135.8	8.5	11.4
4	4	31 May	Rest	0.03	20.94	0.2	148.5	4.95	15.08	35.5	108.2	35.3	40.3
	4	1 June	17.5	2.47	18.13	17.4	127.5	5.39	14.50	38.0	102.2	20.6	25.3
	4		28	4.19	16.25	29.4	114.4	6.14	13.53	42.9	95.0	13.4	19.4
	4	2 June	42	4.60	15.22	32.3	106.8	6.54	13.01	46.0	91.2	13.5	15.6
	4		52	4.98	13.27	35.0	93.2	6.64	10.85	46.5	76.1	11.5	17.1
	4		58	4.78	12.45	33.6	87.3	6.54	10.73	45.9	73.5	12.3	13.8
	4	3 June	66	4.36	13.21	30.6	92.6	6.33	10.04	44.4	70.5	13.8	22.1
	4		72	5.13	10.45	36.2	73.5	6.35	8.72	44.5	60.7	8.3	12.8
5	10	13 July	Rest	0.03	20.94	0.2	148.8	5.96	13.77	42.3	97.6	42.0	51.2
	8	14 July	19	3.07	17.53	22.2	122.7	6.38	12.73	45.5	90.6	22.3	32.1
	10	15 July	31	4.32	15.50	30.7	110.0	6.98	11.91	49.6	84.4	18.9	25.6
	7	16 July	54	4.98	12.83	35.2	90.8	6.88	10.23	48.5	72.1	13.3	18.7

[1] ΔpCO_2 and ΔpO_2 are defined as the difference between ambient and alveolar pO_2 or pCO_2.

above 36 mm. Hg. An increase of carbon dioxide beyond 36 mm. Hg failed to decrease ΔpO_2 and a level is reached where the law of diminishing returns applies (fig. 6).



mm. Hg.			
Ambient Air		Alveolar Air	
pCO_2	pO_2	pCO_2	pO_2
0.2	150.1	40.8	100.4
9.2	140.5	39.3	103.7
17.3	131.20	42.3	98.5
27.5	119.1	43.5	95.0
34.3	111.0	46.4	92.5
42.6	101.5	50.4	89.3
0.2	150.1	41.9	103.0
5.3	143.4	—	—
12.6	134.3	40.4	100.0
22.3	123.7	42.2	95.2
28.8	116.3	44.1	92.9
34.1	109.6	46.3	89.0
40.0	102.8	49.3	88.3
46.2	95.2	55.8	81.4
0.2	150.1	41.1	98.1
15.9	—	41.4	—
31.0	—	46.8	—
38.8	—	50.7	—
48.2	—	56.7	—
0.2	148.5	35.5	108.2
17.4	127.5	38.0	102.2
29.4	114.4	42.9	95.0
32.3	106.8	46.0	91.2
35.0	—	46.5	—
33.6	—	45.9	—
30.6	—	44.4	—
36.2	—	44.5	—
0.2	148.8	42.3	97.6
22.2	122.7	45.5	90.6
30.7	—	49.6	—
35.2	—	48.5	—

Note All alveolar points are averages of from 4 - 10 subjects

FIG. 5. Effects of carbon dioxide on alveolar air at various oxygen concentrations.

TABLE 3. *Effects of increased ambient carbon dioxide on gas equilibria in blood*

EXPERIMENT NO.	DATE	TIME	AMBIENT AIR		ALVEOLAR AIR		BLOOD				PLASMA	
			pCO_2	pO_2	pCO_2	pO_2	O_2 cont.	O_2 cap'y	HbO_2	CO_2 cont.	CO_2 cont.	pH
			mm. Hg		*mm. Hg*		*vol. %*	*vol. %*	*% sat.*	*vol. %*	*vol. %*	
4[1]	6/6	0830	0.2	149.0	39.2	101.3	18.68	19.63	95.3	47.8	58.6	7.44
	6/1	0800	17.4	127.5	38.0	102.2	19.79	20.85	95.0	48.4	58.9	7.45
	6/2	0800	32.3	106.8	46.0	91.2	18.71	20.22	92.6	51.2	59.9	7.38
	6/3	0800	32.4	92.6	44.3	70.5	19.24	21.27	90.5	50.2	60.4	7.40
	6/3	1400	36.0	73.5	44.6	61.2	18.16	20.33	89.3	48.9	59.5	7.38
5[2]	7/13	0830	0.2	149.0	42.4	97.3	19.94	20.34	95.5	47.9	58.2	7.40
	7/16	0700	35.2	90.8	48.5	72.1	18.44	19.93	92.5	53.3	64.6	7.38

[1] Average of 4 subjects.
[2] Average of 5 subjects.

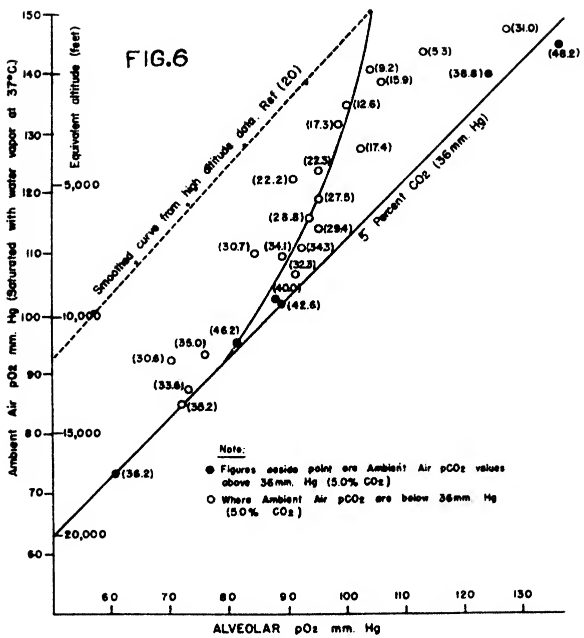
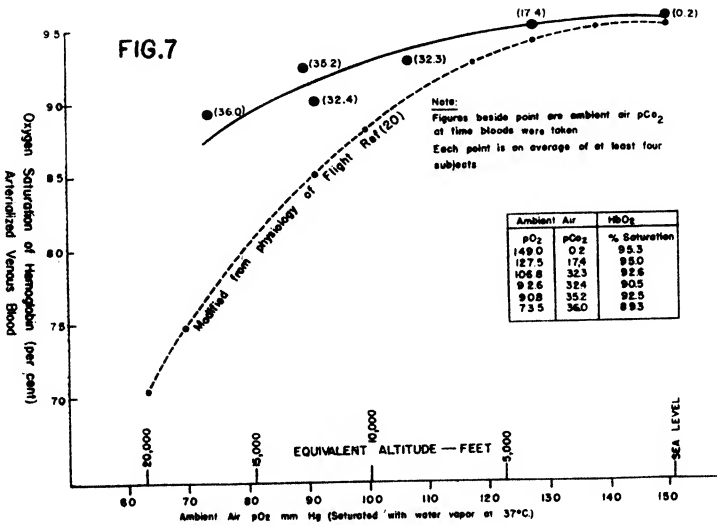


FIG. 6. Effects of carbon dioxide on oxygen pressure in alveoli at various ambient oxygen concentrations.



Ambient Air		HbO_2
pO_2	pCo_2	% Saturation
149.0	0.2	95.3
127.5	17.4	95.0
106.8	32.3	92.6
92.6	32.4	90.5
90.8	35.2	92.5
73.5	36.0	89.3

FIG. 7. Effects of carbon dioxide on oxygen saturation of hemoglobin at various ambient oxygen concentrations.

Physiological Data

High concentrations of carbon dioxide in the ambient air impose a physiological stress by raising the alveolar carbon dioxide pressure and thereby reducing the pressure gradient which is so favorable to the unloading of this gas from the

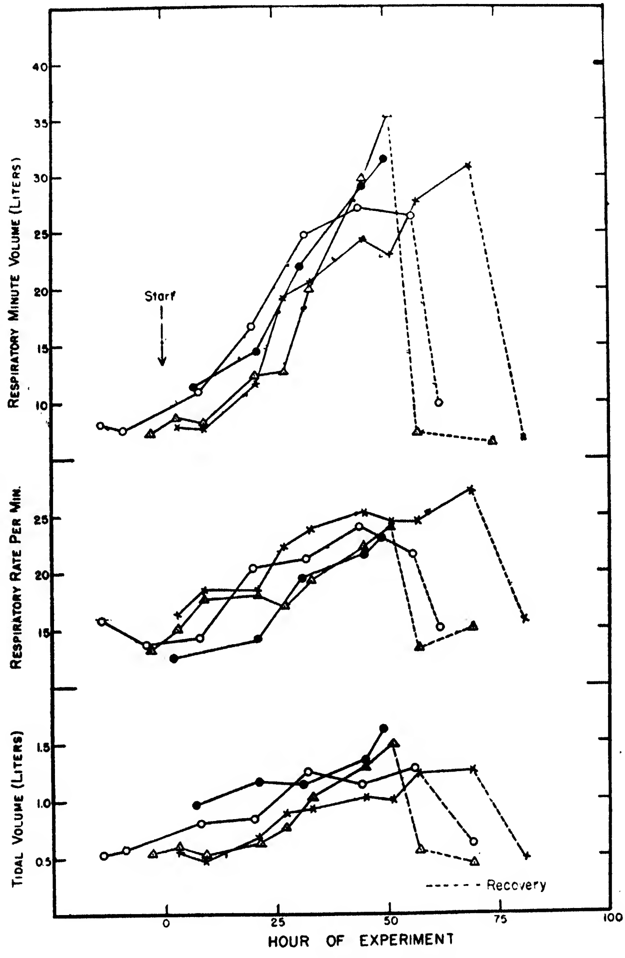


FIG. 8. Effects of carbon dioxide on mean respiration rate, tidal and minute volume. Experiment 3 = △, 4 = x, 5 = ○, and 6 = ●.

pulmonary blood. Hence with heightened alveolar carbon dioxide pressure, it can be assumed that the amount of carbon dioxide unloaded from each unit of blood passing through the alveolar capillaries is reduced. To overcome the barrier imposed by the high ambient carbon dioxide the organism responds by increasing pulse rate, respiratory rate and tidal volume. The pulse rate response

is probably indicative of an increased cardiac output which augments the rate of carbon dioxide transport from the tissues to the lungs, while the respiratory response ensures a more effective removal of carbon dioxide from the alveoli.

Respiration. The most prominent physiologic response to the altered oxygen and carbon dioxide concentrations was the change in respiration. In the course of each experiment, the respiratory rate and the tidal volume approximately doubled and the minute volume was two to three times its normal value (figure 8). It was found that an increase in normal ventilation minute volume of over 300% can be maintained for many hours without serious or persistent effects. Some subjects complained of soreness of the respiratory musculature at the end of the experiments but this symptom disappeared within one or two days.

The increase in respiratory minute volume produced by 3% carbon dioxide was of the order of one and a half times normal, compared with a two- to three-fold increase brought about by 5% carbon dioxide.

Pulse rate. A characteristic mean increase of approximately 10 beats per minute over the normal resting rate occurred when the carbon dioxide concentration reached 5% (fig. 9). That this increase was in response to the increased carbon dioxide pressure rather than to the lowered ambient oxygen pressure was proved by experiment 3, in which the rising carbon dioxide concentration was accompanied by a similar rise in pulse rate, although the oxygen concentration was maintained between 19 and 21%. Figure 9 also shows an approximate difference of 10 beats per minute at equivalent carbon dioxide concentrations between experiments 3 and 4, and 5 and 6. This difference is attributed to effect of temperature on pulse rate. Experiments 3 and 4 were carried out at Effective Temperatures of 85 and 88°, and experiments 5 and 6 at 75 and 60°, respectively (fig. 1–4). Regardless of the effect of temperature on pulse rate, a rise always accompanied an increase in carbon dioxide concentration.

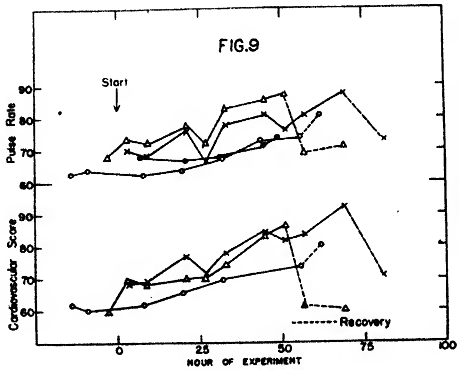
Pulse rate response to exercise paralleled the increase in carbon dioxide (fig. 9). This finding, as in the case of the resting pulse rate, cannot be attributed to the decreased ambient oxygen pressure. It will be noted that in experiment 5 there was a sharp rise of about 8 points in the C. V. S. obtained two hours after the chamber was opened and ventilation with outside air started (recovery point)[2]. In other experiments the C. V. S. was not ascertained until 6 to 9 hours after exposure to outside air. It appears therefore that the exposure to outside air results in a rise in the C. V. S. (two-hour measurement) followed by a fall to pre-experimental level at the end of 6 to 9 hours. Explanation of this rise is difficult, but it can be said that the return to outside air was the most drastic change to which the subjects were exposed, and which in part is reflected by the increased pulse rate response to exercise.

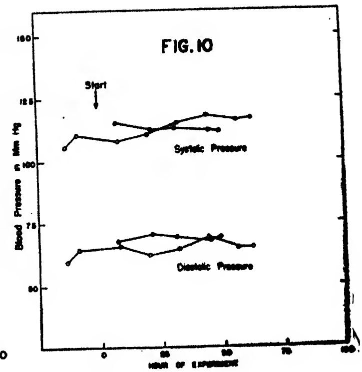
Blood pressure. There were no characteristic changes in blood pressure (fig. 10). In two of the experiments, both the systolic and diastolic pressures showed a tendency to increase but pulse pressure did not change.

Body temperature. Rectal and oral temperatures require separate considera-

[2] The period of re-exposure to outside air.

tion (fig. 11). In experiments 3 and 4, involving few subjects, rectal temperatures were taken, while in experiments 5 and 6 convenience dictated the use of oral thermometers. In the control observations oral temperatures were, as has been commonly observed, roughly one-half degree lower than rectal temperatures. In experiments 3 and 4, the rectal temperatures rose within 5 to 10 hours after the





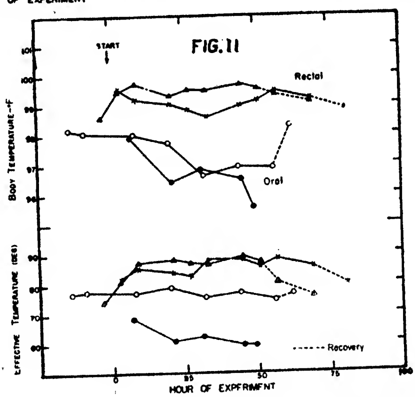


FIG. 9. Effects of carbon dioxide on mean pulse rate and cardiovascular score. Experiment 3 = △, 4 = x, 5 = ○ and 6 = ●.

FIG. 10. Effects of carbon dioxide on mean systolic and diastolic pressure. Experiment 5 = ○ and 6 = ●.

FIG. 11. Mean oral and rectal temperatures and compartment Effective Temperatures. Experiment 3 = △, 4 = x, 5 = ○ and 6 = ●.

start of an experiment to higher levels and dropped almost as rapidly at the close of an experiment. This rise is attributed to the high Effective Temperatures of the experimental chamber (88 and 85°). On the other hand, experiment 5 (Effective Temperature, 75°) and experiment 6 (Effective Temperature, 60°) imposed a heat conservation problem as indicated by the considerable decrease in oral temperature. The subjects were inadequately clothed, particularly in experiment 6, and felt cold. The fall in oral temperature may in part be due to

mouth breathing which many subjects found necessary at high ambient carbon dioxide concentrations as well as to the increased heat loss due to hyperventilation.

TABLE 4. *Subjective symptoms with respect to the subjects, recorded as a fraction (personnel affected/personnel interviewed)*

	HOUR OF TEST	AMBIENT AIR CO_2	AMBIENT AIR O_2	CEREBRAL FULLNESS	HEADACHE[1]	NASAL CONGESTION	NAUSEA	SORE THROAT	DRY THROAT	DRY OR SORE THROAT	FELT GOOD	FELT FAIR
		per cent										
Exp. 5												
Chamber closed	46.5	4.8	13.5		15/35	14/34	5/35			16/34		
	58.5	5.0	12.5		16/34		2/34					
Chamber open	1.5 hr. after test	0.03	20.9		7/34		1/34					
Exp. 6												
Chamber closed	3	0.5	20.5	—	2/20	3/20		1/20	0/20		19/20	1/20
	7	1.3	19.5	1/18	0/18	0/18		0/18	0/18		17/18	1/18
	11	1.9	18.6	1/15	0/13	0/15		0/15	0/15		15/15	0/15
	15	2.4	18.3	1/16	0/16	3/16		1/16	0/16		16/16	0/16
	19	2.8	17.7	1/35	0/35	0/35		3/35	0/35		31/35	4/35
	23	3.3	17.3	1/17	0/17	1/17		0/17	0/17		17/17	0/17
	27	3.9	16.5	0/17	3/17	4/17		1/17	0/17		15/17	2/17
	29	4.2	15.9	1/10	2/10	2/10		0/10	1/10		6/10	4/10
	31	4.4	15.6	1/18	1/18	1/18		0/18	2/18		15/18	3/18
	35	5.0	15.1	0/16	0/16	1/16		0/16	0/16		16/16	0/16
	39	4.8	14.6	0/14	4/14	4/14		2/14	1/14		11/14	3/14
	43	4.8	14.1	4/16	3/16	0/16		0/16	0/16		8/16	8/16
	47	5.1	13.7	1/10	3/10	0/10		0/10	3/10		8/10	2/10
	50	5.2	13.2	6/76	18/76	8/76		6/76	18/76		50/76	23/76[2]
Chamber open	1.5 hr. after test	0.03	20.9	4/76	3/76	—		—	—		—	—

[1] Headaches were for the most part transient and not severe. One man was removed from the chamber because of headache, nausea, vomiting, and a blood pressure rise to 146 mm.

[2] All personnel were in good condition the following morning. Only 3 of 76 individuals complained of malaise at the 50th hour.

Mouth breathing and increased heat loss. The increased rates of ventilation encountered in these experiments caused an increase in rate of heat loss via the lungs. In experiment 6, the subjects were exposed to uncomfortably low temperatures and high humidities. If we assume the temperature to be 61°F., the relative humidity to be 90% and the minute volume of a seated man to be 30 liters, then each man loses about 41.9 cal. per hour through the lungs (21). The

metabolic rate of a seated man weighing 70 kgm. is roughly 100 calories per hour. Hence, under these conditions a man loses about 42% of the heat which he is producing via the lungs. When he is breathing at a more normal rate of 10 liters per minute, he loses only 14% of his heat via this channel. Consequently, thermal insulation adequate for normal breathing becomes inadequate when the rate of ventilation is increased without concurrent increase in rate of metabolism.

In experiments 3 and 4 the subjects breathed humid air at a temperature of over 90°F. Under these conditions the heat loss via the lungs was negligible. Thus, it can be seen that with respect to temperature regulation an increased rate of ventilation without coincident increase in the rate of heat production is a definite liability at low temperatures and is not an appreciable asset at high temperatures unless the relative humidity is low.

Subjective symptoms. Subjective reactions were recorded in experiments 5 and 6 (table 4). The usual symptoms, sore throat, nasal congestion and headache, were experienced about 40 hours after the start of the experiments. In experiment 5, about 40% of the subjects complained of all these symptoms; in experiment 6, dry throats and headaches occurred in 18%, and nasal congestion in about 10%. However, all personnel felt well the morning after the conclusion of each experiment. In the first four experiments, transient headaches occurred frequently after leaving the sealed spaces. A phenomenon of interest was the fleeting smell and taste of ammonia when outside air was breathed following exposure in high carbon dioxide atmospheres.

Psychological

Although there were some unquestionable decrements in test performance in these experiments, the results lead to an interpretation that the losses, when they occur, were not of such magnitude or character as to interfere appreciably with efficiency of personnel performing naval tasks. Five per cent carbon dioxide is not a comfortable concentration for prolonged inhalation, but the data show that its depressing effect is not great. Compensatory mechanisms appear to come into play to mitigate the adverse effects of long exposures. Other conditions, e.g., the extreme Effective Temperature in the early experiments may also have operated to reduce efficiency. Since these conditions would be expected to affect test scores in the same direction as increased carbon dioxide or reduced oxygen, the changes found are maximal if attributed to the major variables of carbon dioxide excess and oxygen deficiency.

The psychological test data have been analyzed to answer five questions.

What changes in performance occurred during the experiments (table 5)? There was no consistent significant effect upon any of the auditory or visual functions measured, nor upon any of the paper-and-pencil test scores, after the subjects practiced. The eye-hand coordination tests showed a slight decline in most cases. Hand dynamometer scores declined 3 to 10% in well-practiced subjects. Even though statistically significant, these changes are believed to be of small practical importance. The amount of body sway consistently increased, and, for the most part, the increases approach statistical significance. Some part, at

TABLE 5. *The difference in performance between the first and last test in the closed chamber*

Each column presents the data for an experiment. Description of the time and conditions of each test period is given at the head of the columns. Items and group means relating to the first test are in the lines labeled (1); those concerning the last test, in (2).

		EXPERIMENT					
		1	2	3	4	5	6
Time to middle of test period from closing chamber	(1)	$1\frac{1}{4}$ hr.	$1\frac{1}{4}$ hr.	$1\frac{1}{4}$ hr.	$1\frac{1}{4}$ hr.	$7\frac{1}{2}$ hr.	6 hr.
	(2)	$31\frac{1}{2}$ hr.	$49\frac{1}{2}$ hr.	$49\frac{1}{2}$ hr.	69 hr.	$57\frac{1}{2}$ hr.	49 hr.
Per cent CO_2 (av.) during test	(1)	0.5	0.6	0.5	0.5	1.1	1.1
	(2)	5.4	6.1	6.0	5.3	4.8	5.1
Per cent O_2 (av.) during test	(1)	20.2	20.4	20.4	20.5	19.6	19.7
	(2)	14.8	14.0	19.8	11.2	12.5	13.4
Number of subjects		4	3	3	4	2	12
Critical flicker frequency (flashes per sec.)	(1)	41.0	43.8		44.5		
	(2)	40.7	40.4		42.9		
	t	<1.0	1.86		1.72		
Dark adaptation threshold (log $\mu\mu$ lamberts)	(1)					3.7	
	(2)					3.7	
	t					0.0	
Pitch discrimination (decile rank)[3]	(1)	2.0	3.0	4.0			
	(2)	4.5	6.3	4.7			
	t	1.35	4.94[1]	<1.0			
Loudness discrimination (decile rank)[3]	(1)	5.2	6.3	4.0			
	(2)	8.2	6.3	4.3			
	t	2.22	0.0	<1.0			
Audiometer, 128 dv. (decibels)[3]	(1)				18.1		
	(2)				13.8		
	t				<1.0		
Audiometer, 1024 dv. (decibles)[3]	(1)				18.8		
	(2)				18.1		
	t				<1.0		
Audiometer, 8192 dv. (decibels)[3]	(1)				−2.5		
	(2)				3.1		
	t				5.02[1]		
Body sway, eyes open (mm. in 2 min.)[3]	(1)	223	500	200	251	174	
	(2)	390	865	244	394	204	
	t	2.75	3.95	1.24	3.07	1.55	

TABLE 5—*Continued*

		Experiment 1	2	3	4	5	6
Body sway, eyes closed (mm. in 2 min.)[3]	(1)	364	714	421	304	216	
	(2)	566	1214	737	550	308	
	t	2.36	1.76	3.12	2.19	2.38	
Railwalking (feet walked in 10 trials)	(1)	47	49	56		46	
	(2)	36	51	49		40	
	t	1.28	<1.0	2.18		1.57	
Hand-arm steadiness (contacts per min.)[3]	(1)	48.4	16.0	54.9	31.8	50.0	
	(2)	133.8	87.8	123.5	98.7	100.2	
	t	15.59[1]	3.72	10.04[1]	2.50	11.76[1]	
Complex tapping (contacts per min.)	(1)	164	170	188	195	159	
	(2)	148	143	179	163	164	
	t	1.40	3.24	<1.0	1.17	<1.0	
Pursuit rotor (contact during 30 sec.)	(1)	24.65	25.90	27.37	26.35	12.85[4]	
	(2)	22.08	25.34	26.31	24.79	16.77	
	t	2.09	<1.0	2.59	1.90	5.31[1]	
Hand dynamometer (kgm.)	(1)	54.5	55.7	56.3	52.6	56.1	56.6[4]
	(2)	49.2	52.0	54.7	49.0	51.8	57.6
	t	2.50	1.58	2.52	2.51	4.26[1]	<1.0
Computation (problems in 6 min.)	(1)	87.8	73.3	116			
	(2)	69.2	78.7	118			
	t	3.48[1]	<1.0	<1.0			
Computation (problems in 10 min.)	(1)				165	167	
	(2)				154	155	
	t				1.0	2.46[1]	
Code test—15 min. (letters per 90 sec.)	(1)	26.7	25.9	32.2			
	(2)	25.1	27.7	32.8			
	t	<1.0	<1.0	<1.0			
Code test—30 min. (letters per 90 sec.)	(1)				28.5	29.3	26.5[4]
	(2)				27.7	29.7	30.2
	t				<1.0	1.05	4.45[1]
Number checking (number correct in 3 min.)	(1)	51.8	55.3	67.0			
	(2)	47.2	55.3	64.3			
	t	<1.0	0.0	<1.0			

[1] Values for *t* and *N* show that the difference between these means is significant at the .05 level of confidence, or lower.

[2] Number of subjects in experiment 5 was 17, except as follows: dark adaptation, 4; body sway, 5; hand-arm steadiness, 15.

[3] A lower score indicates better performance.

[4] The subjects had little or no practice before the experiment.

least, of this increase is a result of heavy breathing. Control of gross body movement is not so severely affected by heavy breathing as is static equilibrium, for railwalking scores showed only an inconsistent trend downward. Of the functions measured, hand-arm steadiness showed the greatest change, but again

TABLE 6. *Comparison of performance at the beginning and the end of the time during which the CO_2 concentration was approximately 5%*

Each column presents the data for an experiment. Description of the time and conditions compared is given at the head of each column. Items and group means of performance relating to the beginning of the time at 5% CO_2 are in the lines labeled (1); those concerning the end of the time at 5% CO_2 are labeled (2).

		EXPERIMENTS		
		4	5	6
Time to middle of test period since closing chamber	(1) (2)	37½ hr. 61½ hr.	34 hr. 58½ hr.	31 hr. 48 hr.
Per cent CO_2 (av.) during test	(1) (2)	4.7 5.2	4.8 4.8	4.1 5.0
Per cent O_2 (av.) during test	(1) (2)	15.6 12.0	15.1 12.5	15.8 13.5
Number of subjects		4	2	12
Critical flicker frequency (flashes per sec.)	(1) (2) t	41.8 43.0 2.43		
Audiometer, 128 dv. (decibels)[3]	(1) (2) t	26.0 10.6 3.37[1]		
Audiometer, 1024 dv. (decibels)[3]	(1) (2) t	16.8 18.4 <1.0		
Audiometer, 8192 dv. (decibels)[3]	(1) (2) t	1.2 6.0 <1.0		
Body sway, eyes open (mm. in 2 min.)[3]	(1) (2) t	260 368 2.82	248 204 1.73	
Body sway, eyes closed (mm. in 2 min.)[3]	(1) (2) t	353 490 2.25	415 308 2.22	
Hand-arm steadiness (contacts per min.)[3]	(1) (2) t	99.1 104.9 <1.0	90.7 100.2 1.58	

Table 6—*Continued*

		Experiments 4	Experiments 5	Experiments 6
Complex tapping (contacts per min.)	(1)	202	153	
	(2)	182	164	
	t	1.38	2.54[1]	
Pursuit rotor (contact during 30 sec.)	(1)	26.39		
	(2)	24.16		
	t	1.44		
Hand dynamometer (kgm.)	(1)	47.6	53.8	58.3[4]
	(2)	48.0	51.8	57.6
	t	<1.0	1.56	<1.0
Computation (problems in 10 min.)	(1)	161	156	
	(2)	162	155	
	t	<1.0	<1.0	
Code test—30 min. (letters per 90 sec.)	(1)	27.9	28.8	29.4[4]
	(2)	28.6	29.7	30.2
	t	<1.0	3.16[1]	1.22

[1] Values for t and N show that the difference between these means is significant at the .05 level of confidence, or lower.
[2] Number of subjects in experiment 5 was 17, except as follows: body sway, 5; hand-arm steadiness, 15.
[3] A lower score indicates better performance.
[4] The subjects had no practice before the experiment.

body movements, resulting from heavy breathing, were obviously an important determining factor.

To what extent does performance change when oxygen percentage gradually decreases and carbon dioxide is maintained at 5% (table 6)? The data of experiments 4, 5, and 6 are relevant to this question. The statistical analysis consisted in comparing performance when the carbon dioxide first reached 5% with performance just prior to return to normal air. In using the data of experiment 4, the small number of cases made advisable the averaging of the scores of the first two and the last two test periods during the 40 hours that the carbon dioxide was at a 5% level.

The results (table 6) show very few changes of significance. Only three of the comparisons show differences significant below the .05 level of confidence, and that for the audiometer (128 double vibrations[3]) is questionable because of mechanical difficulties during the testing. The other two show improvement. The conclusion to be drawn from this analysis is that the subjects were able to maintain their performance levels in all tests during prolonged exposure to 5% carbon dioxide even when the ambient oxygen percentage was decreasing to 12. Further-

[3] Hereinafter, 'dv.'

more, there is evidence in some tests of adaptation to the conditions of the experiments, with consequent improvement in performance during the last 20 hours.

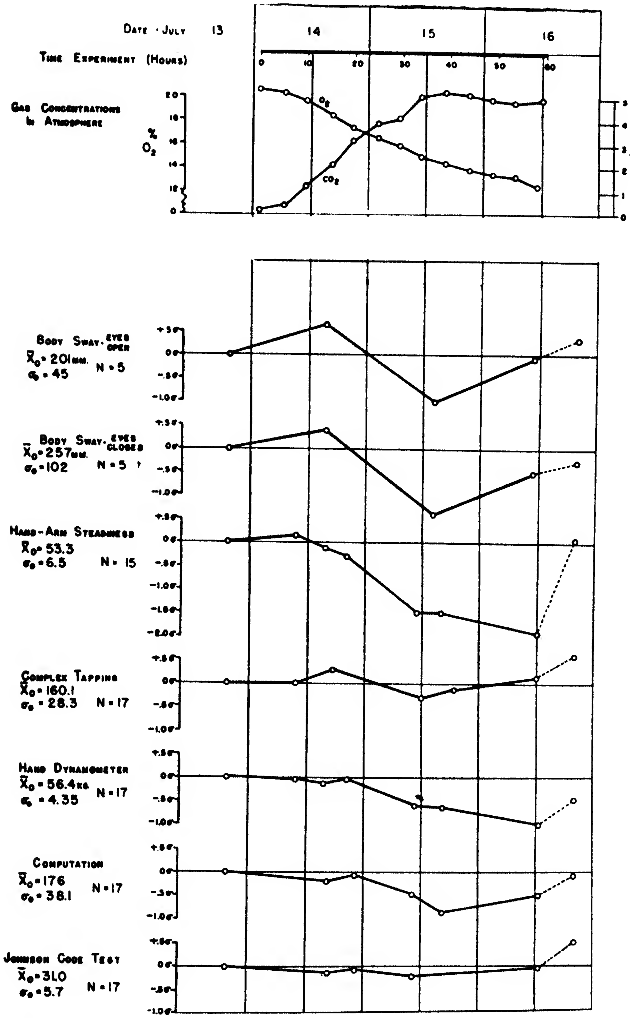


FIG. 12. Effects of increasing ambient carbon dioxide and decreasing ambient oxygen concentrations on psychomotor performance (experiment 5). All ordinates are given in standard deviation units of the score distribution in the last practice period. All scores are plotted so that positive deviation indicates improvement in performance, regardless of the raw score magnitude.

Graphs based on the data obtained from seven of the tests used in experiment 5 are shown in figure 12. All scores are plotted in comparable units, based on the standard deviation of the score distribution of the last practice session, taking

TABLE 7. *Extent and significance of the differences in performance between the last test during rebreathing and the 'recovery test'*

Each column presents the data for one experiment. Description of the conditions compared is at the head of each column. Group means of performance during the last test in the closed chamber are in the lines labeled (1); those for the 'recovery test' are labeled (2).

		EXPERIMENTS				
		1	2	3	4	5
Time of starting test in recovery period		9 hr.	2 hr.	9 hr.	2 hr.	4½ hr.
Highest per cent CO_2 reached		5.95	6.54	6.75	5±	5±
Lowest per cent O_2 reached		14.18	13.45	19.22	10.45	12.21
Number of subjects		4	3	4	4	2
Critical flicker frequency (flashes per sec.)	(1)	40.7	40.4		39.0	
	(2)	41.5	46.3		42.9	
	t	1.90	3.53		2.26	
Pitch discrimination (decile rank)[3]	(1)	4.5	6.3	5.5		
	(2)	3.8	8.3	2.2		
	t	<1.0	3.52	2.18		
Loudness discrimination (decile rank)[3]	(1)	8.2	6.3	4.2		
	(2)	5.2	7.3	1.5		
	t	1.73	<1.0	1.62		
Audiometer, 128 dv. (decibles)[3]	(1)				13.8	
	(2)				9.4	
	t				2.34	
Audiometer, 1024 dv. (decibels)[3]	(1)				18.1	
	(2)				13.8	
	t				2.74	
Audiometer, 8192 dv. (decibels)[3]	(1)				3.1	
	(2)				−1.8	
	t				1.63	
Body sway, eyes open (mm. in 2 min.)[3]	(1)	390	865	294	394	204
	(2)	293	218	115	214	190
	t	1.75	1.78	2.89	4.92[1]	<1.0
Body sway, eyes closed (mm. in 2 min.)[3]	(1)	566	1214	754	550	308
	(2)	505	502	282	244	288
	t	<1.0	1.36	5.55[1]	2.49	<1.0
Railwalking (feet walked in 10 trials)	(1)	36	51	50		
	(2)	35	58	62		
	t	<1.0	<1.0	1.66		

TABLE 7—*Continued*

		EXPERIMENTS				
		1	2	3	4	5
Hand-arm steadiness (contacts per min.)[3]	(1)	133.8	87.8	105.8	98.7	100.2
	(2)	23.8	14.8	38.2	41.5	49.4
	t	13.56[1]	4.16	12.26[1]	2.54	6.37[1]
Complex tapping (contacts per min.)	(1)	148	143	187	163	164
	(2)	182	167	209	213	178
	t	2.43	1.73	1.38	2.38	2.96[1]
Pursuit rotor (contact during 30 sec.)	(1)	22.08	25.34	25.85	24.79	
	(2)	25.12	25.49	27.83	26.44	
	t	1.85	<1.0	3.10	2.12	
Hand dynamometer (kgm.)	(1)	49.2	52.0	53.9	49.0	51.8
	(2)	52.6	55.0	55.0	50.4	54.0
	t	2.64	1.71	1.10	<1.0	2.24[1]
Computation (problems in 6 min.)	(1)	69.2	78.7	111		
	(2)	82.2	74.3	129		
	t	5.73[1]	<1.0	1.74		
Computation (problems in 10 min.)	(1)				154	155
	(2)				184	173
	t				1.73	3.41[1]
Code test—15 min. (letters per 90 sec.)	(1)	25.1	27.7	33.2		
	(2)	28.7	30.2	38.6		
	t	4.92[1]	2.19	2.17		
Code test—30 min. (letters per 90 sec.)	(1)				27.7	29.7
	(2)				31.8	33.7
	t				2.36	6.35[1]
Number checking (number correct in 3 min.)	(1)	47.2	55.3	65.8		
	(2)	53.5	51.7	80.8		
	t	1.58	1.00	6.12[1]		

[1] Values for t and N show that the difference between these means is significant at the .05 level of confidence, or lower.

[2] Number of subjects in experiment 5 was 17, except as follows: body sway, 5; hand-arm steadiness, 15.

[3] A lower score indicates better performance.

the average of this distribution as zero. It will be noted that only hand dynamometer and steadiness scores continued the downward trend during the last 20 hours of rebreathing. For the other tests, the lowest scores occurred between the 30th and 40th hours and subsequently improved.

Is there any advantage in the maintenance of ambient oxygen at approximately 20% if carbon dioxide is allowed to increase to 5%? This question was answered by

comparing the data of the third experiment with those of experiments 1, 2, and 4. Changes in performance between the time the chamber was closed and the time, about 35 hours later, when the carbon dioxide concentration had reached about 5%, were compared under the two conditions of oxygen concentration. The conclusion of this analysis is that 19 to 21% ambient oxygen offers no advantages over oxygen reduced to 15% if the carbon dioxide concentration is concomitantly increased to 5%. In 14 comparisons of changes in test performance, 6 favored conditions of decreased oxygen; no difference was significant at the .05 level of confidence.

TABLE 8. *Improvement with practice on the hand dynamometer test during 50 hours' rebreathing, carbon dioxide concentration being 5% during the last 15 hours*

	EXP. 6	CONTROL GROUP A	CONTROL GROUP B
Number of subjects	12	18	10
First practice score (kgm.)	56.6	52.6	46.5
Sixth practice score (kgm.)	56.8	54.2	49.2
Difference	.2	1.6	2.7
Difference from Experiment 6 group in improvement		+1.4	+2.5
t		<1.0	1.52

TABLE 9. *Improvement with practice on the Johnson Code Test during 50 hours' rebreathing, carbon dioxide concentration being 5% during the last 15 hours*

	EXP. 6	CONTROL GROUP
Number of subjects	15	18
First test score (letters per 90 sec.)	26.6	26.7
Fourth test score (letters per 90 sec.)	29.8	30.9
Improvement	3.2	4.2
Difference from Experiment 6 group in improvement		+1.0
t		1.18

To what extent does performance improve on return to normal air? This is an important question since there is a decrement in some tests, (table 5) and it is important to know whether the unfavorable reactions persist. Table 7 compares performance in the last test period during rebreathing with that in the recovery period (fourth to fifth hour). Of 57 differences, 52 show some average improvement in performance during the recovery period and 12 are significant at the .05 level of confidence or lower.

What is the effect of the experimental conditions on expected improvements from practice (tables 8, 9, and fig. 13)? In experiment 5 the subjects had only two practice sessions with the pursuit rotor before closing the experimental chamber. They had four more practice periods while the chamber was closed, and one in

the recovery test period. The learning curve for the group, compared with three other groups tested with the same apparatus and procedure (fig. 13), suggests that the effect on pursuit rotor learning when living in an atmosphere where concentration of carbon dioxide builds up to 5%, is roughly equivalent to that of living in an atmosphere where the Effective Temperature is above the comfort zone (22). The subjects in experiment 6 had no previous practice on the hand dynamometer or the code test. Their improvement (tables 8, 9) is slightly but not significantly less than that of groups learning under normal conditions. Although there is some indication in these data of interference or inhibition imposed by the experimental conditions during the learning period, the effect is not

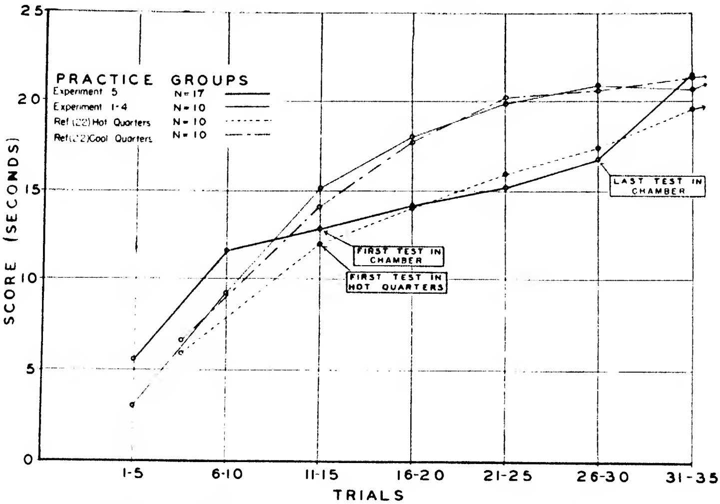


FIG. 13. Pursuit rotor learning under several experimental conditions. Each curve covers a period of four days, with two, five-trial practice periods each day in most cases. The subjects of experiments 1—4 and the 'cool quarters' group were learning under essentially normal conditions. The 'hot quarters' group lived in an environment where the Effective Temperature remained above 85°.

greater than that found in well-practiced performance, when judged by the critical ratio of the changes.

SUMMARY

1. In six experiments men breathed recirculated air for periods of 35 to 72 hours in sealed spaces of such size as to provide 500 cu. ft. of air volume per man.

2. Exposure in atmospheres of carbon dioxide concentrations up to 5% and reduced oxygen concentrations as low as 12% did not seriously impair the physical condition and efficiency of the subjects as evaluated by biochemical, physiological, and psychological tests. Minor symptoms of headache, nasal congestion, and dryness of the throat quickly disappeared when outside air was breathed.

3. In an atmosphere of 5% carbon dioxide and 12% oxygen, healthy men are able to maintain an adequate oxygen pressure in the lungs, blood and tissues because an increase in respiratory minute volume (hyperventilation) and an increase in pulse rate (circulation) prevent a corresponding reduction in oxygen concentration in lungs and blood despite the decrease in ambient oxygen from 21 to 12%. Consequently, in long exposures to atmospheres of high carbon dioxide content (5%) it is not necessary to maintain the oxygen concentration of the recirculated air at the normal value.

4. Concentrations of carbon dioxide much above 5% are not well tolerated. This value appears to be a limiting level for healthy young men if exposures are prolonged.

5. Under these conditions the carbon dioxide output was found to be 0.326 l/min. STP (0.69 cu. ft. per man hour) and the oxygen consumption was 0.387 l/min. STP (0.82 cu. ft. per man hour).

The completion of this project was made possible only by the skilled assistance and perseverance of the following men: V. Broom, H. Collison, L. Hayward, H. Hinshaw, S. Hollander, A. Leggett, W. Platt, J. Shaner, C. Spear, C. Stevens, T. Watson and L. Williamson.

We wish to acknowledge the assistance of investigators from the Naval Research Laboratory, Anacostia, D. C., and the Medical Research Department, New London, Connecticut.

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REPEATED DETERMINATIONS OF PLASMA VOLUME, BLOOD VOLUME AND TOTAL AVAILABLE FLUID IN A GROUP OF NORMAL TRAINED DOGS

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Received for publication October 9, 1947

Cruickshank and Whitfield (1) recently have reported results obtained using cats, which indicate that after an initial intravascular injection of T-1824 there is a period of rapid loss of the dye from the blood. They suggest that unless some means, such as a previous saturating dose of T-1824 (presumably to block the reticulo-endothelial system), is employed, there is a possibility of the value obtained for plasma volume being too high. From this it follows that if repeated determinations of plasma volume are made by the reinjection of T-1824 within a short period of time, the value obtained for plasma volume by the first determination should be significantly higher than that by the later determinations.

This report presents the results obtained when repeated determinations of plasma volume, blood volume and total available fluid were made in dogs. In carrying out these determinations no efforts were made to block the reticulo-endothelial system by a saturating dose of T-1824 prior to the initial determination.

METHODS

The animals used in these experiments were 6 normal, fully grown male dogs. These animals were fasted 16–20 hours before each experiment and they were trained to lie quietly upon the operating table during the course of an experiment. They had been used over a period of one and one half years to study the effects of some anesthetic agents upon the various fluid compartments of the body (2), and in this period some 104 determinations of normal values were made. A summary of these values is presented in table 2.

Plasma volume was estimated by the use of the dye T-1824 and total available fluid by sodium thiocyanate (3). The two materials were injected intravenously simultaneously as described by Gregersen and Stewart (4), and the concentrations of the dye and thiocyanate in subsequent blood samples were determined using the Evelyn colorimeter with filters 635 for the T-1824 and 490 for the thiocyanate.

In each experiment a control sample of blood was taken before the injection of the dye and thiocyanate, and blood samples were taken at 25, 35, 45 and 55 minutes, or 30, 40 and 50 minutes after the injection. On completion of this determination a second mixture of dye and thiocyanate was injected intravenously and the determination repeated. This experimental procedure was repeated at widely separated time intervals in the same animals, to avoid the effect upon the disappearance curve of the newly injected dye of any dye re-

Table 1

DOG	WT.	% CELL VOL. 1st	% CELL VOL. 2nd	% CELL VOL. % diff.	PLASMA VOL. 1st	PLASMA VOL. 2nd	PLASMA VOL. % diff.	RED CELL VOL. 1st	RED CELL VOL. 2nd	RED CELL VOL. % diff.	TOTAL AVAIL. FLUID 1st	TOTAL AVAIL. FLUID 2nd	TOTAL AVAIL. FLUID % diff.
	kgm.				*cc.*	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*	
1	24.6	46.4	46.1	−0.65	1227	1250	+1.88	1062	1068	+0.56	7407	7272	−1.83
1	24.6	43.2	43.8	+1.39	1258	1212	−3.66	957	945	−1.25	9195	9091	−1.13
1	23.4	42.7	41.4	−3.05	1257	1266	+0.72	937	894	−4.58	8000	7952	−0.60
2	17.7	37.5	36.7	−2.14	1143	1176	+2.88	686	682	−0.58	6061	6107	+0.76
2	17.7	38.1	37.1	−2.62	1087	1130	+3.96	668	667	−0.15	4969	5128	+3.20
2	17.7	37.8	37.4	−1.06	1143	1163	+1.75	693	695	+0.29	6400	5517	−13.80
2	17.3	38.6	38.2	−1.04	1250	1194	−4.48	776	741	−4.51	6202	6033	−2.72
3	22.3	44.6	43.1	−3.36	1170	1143	−2.31	942	866	−8.08	6250	6504	+4.06
3	22.7	39.9	39.0	−2.26	1266	1290	+1.89	841	823	−2.14	6061	6375	+5.18
3	22.3	39.9	38.8	−2.76	1195	1212	+1.42	793	769	−3.03	6038	5714	−5.37
4	17.5	44.7	44.6	−6.22	1176	1197	+1.79	952	982	+3.16	6038	6226	+3.12
4	17.1	46.9	46.1	−1.71	941	976	+3.72	831	835	+0.48	4819	5000	+3.75
4	16.9	44.7	44.9	+0.45	930	962	+3.44	752	784	+4.25	5141	5208	+1.31
5	16.4	35.8	35.3	−1.39	941	920	−2.23	525	502	−4.38	4819	5000	+3.75
5	15.7	37.3	26.7	−2.20	1170	1190	+1.71	439	433	−1.37	5229	5229	0
5	16.4	42.3	41.7	−1.42	962	952	−1.04	705	681	−3.40	4598	4663	+1.42
6	20.0	46.0	45.1	−1.96	909	952	+4.74	773	781	+1.04	5215	4969	−4.72
6	20.2	47.1	46.5	−1.28	1000	976	−2.40	890	848	−4.73	5780	5839	+1.02
Mean				−1.5155			+0.7655			−1.5788			−0.1444
Standard Error of Mean				0.288			0.6543			0.7279			1.1448
'T'				5.262			1.1699			2.1690			0.1349
Significance				S			N.S.			S			N.S.

The 'T' value for P = 0.05 and N = 17 is 2.11.

Table 2. *Values obtained from the different body fluid compartments in the normal animals used in this series, compared with those normal values reported in the literature.*

REF. NO.	ANIMAL & NOS.	NO. OF DETNS.	METHODS USED	PLASMA VOL. MEAN & RANGE	RED CELL VOL. MEAN & RANGE	TOTAL BLOOD VOL MEAN & RANGE	TOTAL AVAIL. FLUID MEAN & RANGE
				cc./kgm.	*cc./kgm.*	*cc./kgm.*	*cc./kgm.*
This rept	6 dogs	104	T-1824 NaSCN	56.3 (40.6–75.7)	40.3 (27.6–66)	96.6 (69.8–137)	312.4 (240–380)
4	15 "	72	T-1824 NaSCN	54.7 (35–65)			310.26 (230–425)
12	50 "	50	T-1824	48.9 (41.2–51.7)	43.9 (36.4–54.6)	92.7 (84–97.3)	
13	29 "	29	T-1824	54	25	79	
14	106 "	106	T-1824	48.3 (31.8–69.6)	34.9 (21–49.2)	83.2 (60–107.5)	
15	dogs		T1824 CO			99 99	
16	16 "	16	T-1824 CO	55.4 55.1	39.8 40.3	95.2 ±12.4 95.4 ±12.0	
17	9 "	9	T-1824 NaSCN	41.1 (34–48)			259.6 (211.9–297.6)
18	6 "	6	Dye (?) Radioactive iron	46.2 (34.7–60.6)	31.5 (23–35.5)	77.7 (65.7–94.6)	
19	33 humans	33	T-1824 NaSCN	44.8 (36.9–54.7)	36.8	81.6 (59.2–103.7)	188 (163–246)
16	9 "	9	T-1824 CO	45.5 45.3	35.0 34.9	80.5 ±8.6 80.2 ±5.5	
20	59 "	59	T-1824 NaSCN	36.7			226
21	10 "	10	T-1824	48.8 (41.5–54.7)			
22	6 "	6	Radioactive phosphorus	42.7 (30.13–63.37)	30.96 (21.96–41.57)	73.6 (53.7–103.7)	
23	32 "	64	T-1824 NaSCN	45.2 (35.3–55.9)	33.1		235 (202–263)

maining in the vascular system from the previous estimation. The initial control blood samples were always clear of dye.

The details of estimating the concentration of dye and thiocyanate have been described previously (2). The plasma sample was measured into the colorimeter tube and the estimation of the concentration of both dye and thiocyanate carried out in the same tube. The usual precautions were taken to avoid hemolysis, but a correction for any possible hemolysis was carried out (5) at the time of estimating the concentration of T-1824. The methods used in the determination of the per cent cell volume (packed cell volume) have been adequately described elsewhere (2, 4).

RESULTS

Table 1 presents the absolute values for plasma volume, red cell volume, per cent cell volume and for total available fluid in a series of 18 experiments.

Statistical analysis of the difference between the first and second sets of values has been made applying Fisher's *t* test (6). From this analysis it is seen that no significant difference existed between the first and second determinations of plasma volume or of total available fluid. However, there was a significant decrease found between the first and second determinations of per cent cell volume, red cell volume and total blood volume (total blood volume figures are not presented in the table).

In table 2 the range and mean of the 104 normal readings obtained in the 7 dogs which were used in these experiments are given and compared with some of the values reported in the literature for plasma volume, blood volume and total available fluid.

DISCUSSION

Cruickshank and Whitfield (1) reported that the disappearance curve of T-1824 in cats may be divided into 3 portions: *a*) a short period of the order of one minute for mixing, *b*) a period of absorption during which there is a rapid loss of dye from the blood, lasting from one to 10 minutes, and *c*) the disappearance curve of the dye upon which *a*) and *b*) are superimposed. On the basis of these findings they have suggested that unless precautions, such as a previous saturating dose of T-1824 or an injection of India ink, are taken to block the reticulo-endothelial system, there is a possibility of the values for plasma volume obtained with T-1824 being too high. This results from the dye concentration obtained by extrapolation being too low, and thus these authors advocate a previous saturating dose of T-1824 20 minutes before the injection of the dye mixture used in determining the plasma volume.

In the series reported here, in dogs in which no precautions were taken to block the rapid loss of dye from the blood stream, no significant differences were found between the two determinations of plasma volume. A significant difference would have been expected if the conclusions of Cruickshank and Whitfield applied in the case of the dog. It is also of interest that there was no significant difference observed between the two determinations of total available fluid (thiocyanate space).

An examination of the figures in table 1 indicates that while there was some variability in the volumes of the various fluid compartments in the same animals when the determinations are carried out on widely separated days, these volumes were quite constant over the period of a few hours involved in making the two determinations of plasma volume, etc.

The significant decrease in per cent volume found in the second determination is responsible for the decreases observed in red cell volume and in total blood volume, since the per cent cell volume and the plasma volumes are the basis for the calculation of the red cell and total blood volumes.

The cause of this reduction in per cent cell volume is rather obscure. It might possibly result from the further relaxation on the part of the animal as the experiment progresses, or it might possibly be the result of some action of the thiocyanate. However, Hemingway, Scott and Wright (7) have reported that hemoglobin values fall in dogs on repeated sampling of blood. Mole (8) has reported that the variation in hemoglobin on repeated sampling of blood has a standard deviation of 0.54 volumes CO (0.46 volumes after allowing for diurnal variation). This would mean that in repeated hemoglobin samples there must be nearly 8% difference between the samples before the difference can be attributed to anything but experimental error.

Gibson and Evans (9) noted a similar decrease in per cent cell volume in humans on repeated sampling which tended to level off in about 20 to 30 minutes. Bonnycastle (10) examining this question in dogs repeated the procedure of redetermining the blood volume described above, using either saline alone or saline and thiocyanate in amounts equal to the volume of fluid used in determining total available fluid. It was observed that the initial injection of thiocyanate and saline led to a similar decrease in per cent cell volume as did the injection of saline alone. The second injection, however, resulted in a greater fall in per cent cell volume than did the second injection of saline. In both cases the decrease in per cent cell volume was greater than when no saline or thiocyanate was given, and the same size blood samples taken at the same periods. In this case the per cent cell volume leveled off after about 30–40 minutes.

Reeve and Armin (11) have reported the results of repeated determinations of plasma volume in humans by reinjection of T-1824. The values they obtained by the first and by the second injection of dye do not differ by more than the error of the method. No attempt was made in these experiments to block the reticulo-endothelial system.

Thus it would appear that in so far as the disappearance of the dye T-1824 is concerned, it apparently behaves similarly in man and the dog, no saturating dose of it being required before utilizing the dye for the estimation of plasma volume.

In table 2 the mean value and the ranges of 104 normal determinations on these dogs are compared with similar values from the literature. It is seen that these values agree reasonably well with those reported by other workers. Some of the reported human values are included for comparison.

SUMMARY

1. In these experiments in dogs using both T-1824 and sodium thiocyanate, there is no evidence of erroneously high values of plasma volume in the first determination, as a result of the immediate absorption of the dye by the reticulo-endothelial system.

2. In the determination of plasma volume in dogs with T-1824, no pre-injection of the dye is required.

3. The 104 normal values obtained in 7 dogs for plasma volume, blood volume and total available fluid agree satisfactorily with those reported in the literature.

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A RELATIONSHIP BETWEEN THE BODY TEMPERATURE AND THE BLOOD PRESSURE IN THE CHICKEN[1]

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Received for publication September 9, 1947

This laboratory has previously called attention to a relationship between the body temperature and the systemic arterial blood pressure observed in the turtle, a cold-blooded animal (1). These findings suggested the possibility that such a relationship might also exist between the body temperature and the arterial pressure in warm-blooded animals. In this report we discuss blood pressure and heart-rate data obtained on chickens at their normal body temperature of about 42°C., and the changes observed as the body temperature was changed by cooling or warming.

METHODS

We used 22 chickens (*Gallus domesticus*) of the White Rock or White Leghorn breeds, ranging in age from 6 to 16 weeks and weighing 450 to 1200 grams.

In order to be able to take direct arterial blood-pressure readings it was necessary to put the animals into a quiescent state. This is accomplished easily by placing the chicken on its side on an animal board with its head tucked under the lower wing. A deeper state of quiescence is obtained by rotating the animal at arm's length several times before placing it on the animal board. In this condition the animal is usually not responsive to the simple procedure necessary for exposure of the ischiatic artery.

In our early experiments intravenous pentobarbital sodium (25 mgm/kilo) was used as the anesthetic agent. Later the anesthetic agent was discontinued for several reasons: *a*) The amount of anesthesia required was found to vary with the body temperature, decreasing as the latter was reduced. Doses which are anesthetic at the normal body temperature may be lethal at reduced body temperature (2). *b*) Hypothermia is itself an effective anesthetic agent (2). *c*) Anesthesia would tend to mask any mechanism which depended for its action on the central nervous system. This latter factor was of extreme importance inasmuch as we have previously demonstrated that in the turtle the body temperature-blood pressure relationship depended upon the integrity of the central nervous system (3).

The feathers were plucked from the skin overlying the proximal end of the femur. A small incision was made over the posterior margin of the proximal end of the femur and the ischiatic artery was found lying in a sheath with its nerve and vein immediately posterior to the bone. The artery was cannulated

[1] Aided by the A. D. Nast Fund for Cardiovascular Research, and in part by a grant from the War Department to the Michael Reese Hospital (Dr. L. N. Katz, responsible investigator).

and the cannula attached to the lead tubing of the Hamilton manometer. A small amount of heparin was used in the cannula to prevent clotting. To record body temperature, a mercury thermometer was inserted deep into the cloaca.

The animals were cooled by placing a bag of crushed ice on the body, and especially under the wing. Warming was accomplished by radiant heat from two 150-watt Spot Reflector light bulbs placed about 30 cm. from the body. The rate of temperature change was about 1° C. per 10 minutes during cooling, and about 1° C. per 8 minutes during warming.

Blood pressures were recorded before any change in body temperature and with each change of 2 or 3° C. One group of animals was cooled to the lower limit of cold tolerance, about 24–25° with our method and rate of cooling, and then rewarmed to the control body temperature. Warming was then continued until exitus which occurred at 45–46° C. In another group of animals no cooling was done. Instead, after a series of control readings at normal body temperatures, the animals were warmed directly until exitus which occurred at about 46° C.

In order to test the reactivity of the cardiovascular system at various body temperatures, epinephrine (1 mgm.) dissolved in 1 cc. of saline was injected into the sciatic vein and the blood pressure was recorded until it returned to normal.

RESULTS

1. *The normal blood pressure of the chicken.* We found the blood pressure of the chicken to be remarkably constant and to fall within a relatively small range. The average blood pressure which we have obtained, so far, in a series of 100 chickens was 135/120 mm. Hg with a range of ± 20. The standard deviation of our series was 11. The pulse pressure averaged 18 mm. Hg and ranged from 5 to 60 mm. Hg. However, 85 of our 100 readings had a pulse pressure ranging from 10 to 25 mm. Hg. In 19 readings the presence of a short length of rubber tubing, about 1 cm. long, between the glass cannula and the lead tubing of the Hamilton manometer may have reduced the pulse pressure somewhat. The scattergram for our series is given in figure 1. Repeated measurements of the blood pressure in the same chicken were found to vary less than ± 10 mm. Hg. Because of this the chicken would seem to lend itself admirably to some types of blood-pressure studies since the variation from animal to animal is small, and the blood pressures usually do not change significantly from reading to reading.

2. *The effect of cooling and rewarming.* (*a*) *Blood pressure.* Placing the animal on the animal board and leaving it immobile for periods up to 2 hours had no effect on the body temperature or arterial pressure of the older and larger chickens. In the smaller animals the body temperature sometimes tended to fall 1–3°, and this was accompanied by no significant change in blood pressure. These and the following data represent the results on unanesthetized chickens.

Ten chickens were actively cooled by means of application of ice packs and then rewarmed by radiant heat. During the cooling phase the blood pressure fell in all 10 experiments, the fall usually being proportional to the degree of body-temperature reduction. When the animals were rewarmed, all but one showed

a progressive rise in pressure toward the control level. During the rewarming phase the blood pressures attained at the various body-temperature levels were

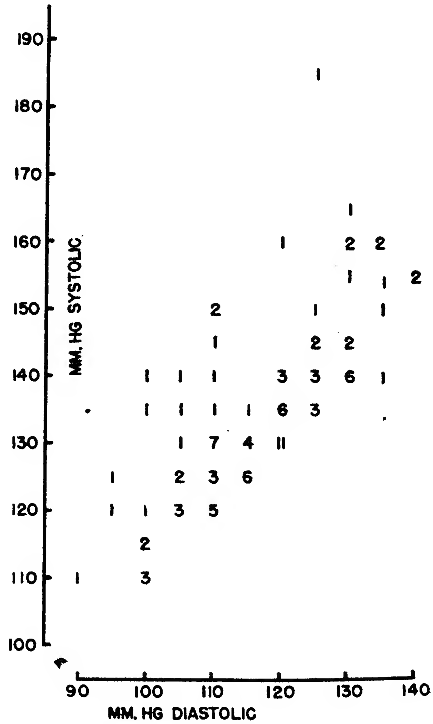

FIG. 1. BLOOD PRESSURE OF 100 RESTING UNANESTHETIZED CHICKENS. Each number represents the number of animals that fall in each group indicated by the ordinates (systolic) and abscissae (diastolic) values. For example, the figure *5* indicates that 5 chickens had blood pressures of $^{120}/_{110}$ mm. Hg.

TABLE 1. *Effect of body temperature change on arterial pressure of the chicken*

TEMPERATURE	DIASTOLIC BLOOD PRESSURE	
	During cooling	During rewarming
°C.	*mm. Hg*	*mm. Hg*
44		96 ↑
41	114 (control)	112
35	106	105
30	92	91
25	↓ 78	

on the average remarkably similar to those obtained during cooling (table 1). When the normal body temperature of about 41.5° C. was reached, all but two had returned to within 10 mm. Hg of the control blood-pressure level. A typi-

cal response to temperature change is shown in figure 2. Averages for the diastolic blood pressure of the entire group are given in table 1. The systolic pressures were in general about 15 mm. Hg above the diastolic.

The warming was continued so that the body temperature was raised above the normal level in 6 of the chickens which had been cooled. In 4 others fever was induced directly without previous cooling. In these the blood-pressure changes were less consistent. In all 10 animals the blood pressure tended to fall as the body temperature was increased. During the early phase of the warming 3 animals showed a transient rise in pressure of about 10 mm. Hg above the control values. In the other 7 a fall in pressure was seen soon after fever temperatures were obtained. As the animals reached body-temperature levels of about 45° C. the blood pressure fell sharply in all and they soon expired.

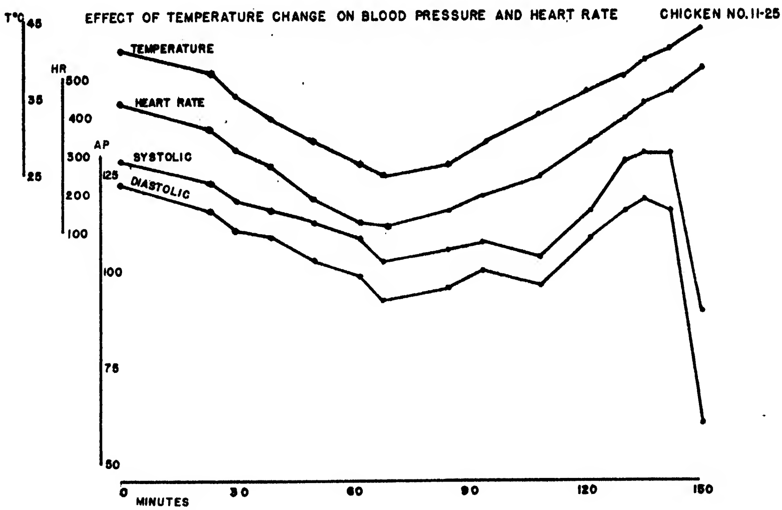

FIG. 2

(b) *Heart rate.* The heart rate varied directly with the body temperature of the animals. These heart rates during cooling and warming are given in table 2.

It will be noted that the heart rate was on the average somewhat slower at the same temperatures during the cooling phase than during the warming phase. It is of interest also that the heart rate continues to increase regularly when the body temperature is raised above normal, while the blood pressure usually does not rise and always begins to fall when temperatures around 45° C. are obtained. This would appear to eliminate the heart rate as a primary factor in the mechanism which binds the arterial pressure values to the level of body temperature.

3. *The response to epinephrine at various body temperatures.* In our early experiments, injections of epinephrine were given just before death of the animal in order to determine whether or not the vessels were still capable of res-

ponding to a pressor stimulus. In every case there was a typical response with a marked rise in arterial pressure. This occurred even in animals a minute or two before death when the blood pressure was falling rapidly after the body temperature had reached toxic levels of 23–25° C. during cooling, or 45–46° C. during warming.

In order to test the possibility that changes in the reactivity of the blood vessels might be responsible for the changing blood pressure at different body temperatures, a standard injection of 1 mgm. of epinephrine was given intravenously. In this way it was hoped to measure any large changes in vascular reactivity. The possibility that vascular reactivity might not be indicated by the response to epinephrine was entertained, but no other adequate method presented itself. The average response in terms of change in arterial pressure from that existing before injection is given in table 3.

TABLE 2. *Effect of body temperature change on heart rate in the chicken*

TEMPERATURE	HEART RATE	
	During Cooling	During Rewarming
°C	*beats/min.*	*beats/min.*
45		550↑
41	375	390
35	290	340
30	165	204
25	↓108	

TABLE 3. *Response to epinephrine at various body temperatures*

BODY TEMPERATURE	BLOOD PRESSURE RISE ABOVE PRE-INJECTION VALUES	
	Systolic	Diastolic
°C	*mm. Hg*	*mm. Hg*
44	70	55
42	70	55
35	62	50
30	65	55

It can be seen from the table that there was no significant change in the blood pressure response to epinephrine from that observed at normal body temperature in either the hypothermic or febrile chickens. Therefore, there is no significant difference in the vascular responsiveness to the pressor stimulus applied. It was of some interest to note in two instances, in which the arterial pressure was very low preagonally, of the order of 20/15, that injection of epinephrine caused a greater rise in pressure than indicated in table 3, viz., of the order of 140/140. It would thus seem that even at a time when the arterial pressure is falling rapidly to zero, the inability to maintain pressure would appear to lie outside the realm of the responsiveness of the vascular tree itself.

The time required for the blood pressure to return to the pre-ephinephrine level was about 6 minutes when the animals were at 42° C., and was not significantly changed at 44° C. However, cooling of the animals caused an increase in the time required. At 35° an average of 19 minutes elapsed, and at 30°, 10 minutes was required.

DISCUSSION

In this study we set out to discover if a relationship could be established between the body temperature and the arterial pressure of a warm-blooded animal, the chicken, similar to that which has been noted in the frog (4) and in the turtle (1, 5). Such a relationship appears to hold, comparable in general to that in the poikilotherm, except that the normal pressure level seen in the chicken is much higher, being about 135/120 mm. Hg. Lowering of the body temperature results in a progressive fall in arterial pressure with a return to control values as the animal is rewarmed. Warming the animal above the normal body-temperature level does not necessarily cause a further rise in pressure and usually leads in time, at higher temperature levels, to a fall in pressure.

It is worthy of note that the normal blood pressure of the few mammals with which we have had experience in this laboratory, the rat, rabbit, dog and man, all have diastolicpressures in the mature animal, ranging about 80 ± 20 mm. Hg on the average, and body temperatures ranging between 37 and 39° C. (6–8). The chicken, which has a higher normal body temperature, also has a higher diastolic pressure, averaging about 120, a level which would be considered hypertensive in the mammal.

The mechanism of the relationship between the body temperature and the arterial pressure in the chicken is not yet elucidated. However, our epinephrine experiments suggest that the changes in pressure are not due to changes in the reactivity of the blood vessels at various body temperatures, since the response to a standard dose of epinephrine is similar in animals at normal body temperature, animals cooled as low as 30° and those warmed as high as 45° C. It probably is not related to the changes in heart rate seen with body temperature change, since at temperatures above 42° C. the heart rate increases consistently while the blood pressure may remain unchanged or even fall. Its possible dependence on changes in cardiac output remains to be determined, although it may be inferred from the large changes in heart rate that significant changes in cardiac output must also occur.

A clue to the mechanism of the temperature-pressure relationship is obtained in our experiments on the turtle. By sectioning the cervical spinal cord or destroying the brain, we have been able to eliminate the temperature-pressure relationship, after which the blood pressure remained unchanged regardless of body temperature change (3). This held despite the fact that changes in heart rate with temperature continued as before the interruption of the higher nervous pathways. Also in the turtle the blood-pressure response to epinephrine was unchanged following destruction of the brain or section of the cord, indicating, as in the present experiments in the chicken, that no measurable

alteration in the reactivity of the blood vessels to epinephrine occurs at changed body temperature (3).

These data support a working hypothesis developed in this laboratory (9) that there is a direct control of the blood pressure by the brain which serves to maintain an adequate supply of blood for the metabolic needs of the body. As the metabolic needs change with changing body temperature, an active adjustment operating through the nervous system is made in the head of pressure driving the blood through the vascular system to the tissues. That the blood-pressure adjustment is not the only one which occurs is clear from our earlier findings that in the chicken, at least, the blood sugar decreases with falling body temperature and increases as the animal is rewarmed (10). The present experiments are similar except that the point of attack is the circulatory system.

The maximal body temperature for which this temperature-pressure mechanism can adjust appears to be the normal body temperature of the animal, about 42° C. in the present case of the chicken. At temperatures higher than this, the mechanism does not continue to function adequately, and this breakdown in function may play a rôle in the death of the animal.

SUMMARY

1. A simple method for measuring the direct arterial pressure of the unanesthetized resting chicken is described.

2. The average blood pressure of the unanesthetized resting chicken is 135/120, with a standard deviation of 11 mm. Hg.

3. Lowering of the body temperature of the chicken causes an immediate progressive fall in the blood pressure. Rewarming causes a rise in pressure with a return to the control blood pressure when normal body temperatures are again achieved.

4. Warming the chicken above the normal body temperature causes variable changes in the arterial pressure at first, but in all cases the pressure finally falls rapidly at about 45° C.

5. The changes in pressure do not appear to be due to changes in the responsiveness of the blood vessels or to changes in the heart rate. It is suggested that they are due to a mechanism residing in the brain.

6. Some implications of these data are discussed.

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TRANSIENT HYPOTENSION FOLLOWING RAPID INTRAVENOUS INJECTIONS OF HYPERTONIC SOLUTIONS

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Received for publication September 23, 1947

During the course of a series of experiments dealing with the use of concentrated plasma and serum in the treatment of oligemic shock in dogs, a peculiar transient lowering of the mean arterial blood pressure was noted and recorded (1, 2). It soon became apparent that this phenomenon was related to multiple factors prominent among which could be included the concentration of the solutes and the speed of administration in relation to the size of the animals. The effect was termed the 'transient depressor effect' of concentrated solutions (1) and the present report is an account of experiments designed to determine the mechanism of its production.

METHODS AND RESULTS

All animals used in these experiments were healthy, mature dogs weighing from 8 to 16 kgm. Sodium pentobarbital anesthesia was used and the trachea was cannulated routinely. Blood pressure was obtained from the carotid artery using a mercury manometer with a float writing point recording on a smoked drum. A heparin solution was used in the blood pressure recording system to avoid the interfering blood pressure effects of sodium citrate during the hypotensive phases. Injection time was recorded by a signal magnet on a drum of constant speed and by the use of a stop watch. Plasma, serum, glucose and sodium chloride solutions were used in strengths that were hypertonic (3–10×) and isotonic to normal blood plasma. A hypertonic albumin solution was also used.

As seen in line 2 of table 1, intravenous injections of 50% glucose in doses as low as 0.83 ml. per kgm. or at rates as low as 0.11 ml. per kgm. per second consistently caused a drop in mean arterial pressure. Even greater lowering of blood pressure resulted from the injection of 10% sodium chloride in comparable volumes and rates (line 3, table 1). However, there was no absolute correlation between the rate and volume of the injection and the amount of depression of the blood pressure. In general, the same effect was observed following rapid injection of concentrated human plasma (line 4, table 1) or a 25% solution of albumin (line 5, table 1).

When the rate of injection of similar doses of the same hypertonic preparations was slowed to less than 0.04 ml. per kgm. per second, only 3 of 70 injections produced a drop in mean arterial pressure exceeding 5 mm. of mercury (lines

TABLE 1. *Effect of rate and site of injection of isotonic and hypertonic solutions on arterial blood pressure*

LINE NUMBER	SUBSTANCE INJECTED	CONCENTRATION	SITE OF INJECTION	NO. OF ANIMALS	NUMBER OF INJECTIONS	VOL. INJECTED	INJECTION RATE	CHANGE IN BLOOD PRESSURE
						ml/kilo	*ml/kilo/sec.*	mm. Hg
1	Sodium chloride	0.9%	Femoral vein	7	23	0.80 –4.43 (1.38)	0.066–0.300 (0.223)	−10[1] to 0 (0)
2	Glucose	50%	" "	19	38	0.83 –2.0 (1.28)	0.11 –0.42 (0.24)	−18 to −122 (−38)
3	Sodium chloride	10%	" "	13	26	0.56 –2.32 (1.19)	0.13 –0.43 (0.27)	−26 to −88 (−52)
4	Human plasma	Normal ×3	" "	10	15	0.83 –2.83 (1.30)	0.07 –0.27 (0.17)	0 to −72 (−30)
5	Albumin	25%	" "	2	4	1.34 –2.68 (1.98)	0.14 –0.18 (0.16)	−22 to −46 (−32)
6	Glucose	50%	" "	12	40	0.66 –2.13 (1.30)	0.010–0.043 (0.025)	+12 to −16 (−1)[2]
7	Human plasma	Normal ×3	" "	8	22	0.66 –1.60 (1.03)	0.014–0.036 (0.025)	+38 to −10 (+1)[3]
8	Human serum	Normal ×3	" "	3	8	0.58 –1.33 (0.93)	0.015–0.024 (0.019)	0 (0)
9	Glucose	50%	Right ventricle	4	7	0.93 –1.67 (1.26)	0.185–0.465 (0.291)	−6 to −42 (−26)
10	"	50%	Left ventricle	4	5	0.93 –1.56 (1.22)	0.093–0.465 (0.240)	−20 to −52 (−34)
11	"	50%	Root of aorta	5	9	0.93 –1.92 (1.24)	0.138–0.465 (0.262)	−10 to −48 (−28) 1 F
12	Sodium chloride	10%	" " "	3	4	1.111–1.85 (1.49)	0.222–0.370 (0.283)	−30 to −50 (−41)
13	Glucose	50%	Abdominal aorta	4	4	2.19 –2.84 (2.41)	0.337–0.568 (0.429)	+10 to +30 (+21)
14	Sodium chloride	10%	" "	5	6	1.14 –6.25 (2.47)	0.227–1.25 (0.50)	+18 to +50 (+33)
15	Glucose	50%	Femoral artery	3	5	1.25 –1.50 (1.28)	0.208–0.301 (0.247)	+12 to +32 (+23)
16	"	5%	Coronary artery	3	5	0.40 –0.70 (0.62)	0.036–0.058 (0.048)	0 to +15 (+8)
17	"	20%	" "	4	5	0.40 –0.71 (0.51)	0.031–0.10 (0.049)	+6 to −15 (−2)
18	"	30%	" "	4	4	0.44 –0.70 (0.55)	0.038–0.058 (0.045)	−10 to −20 (−14) 1 F
19	"	40%	" "	4	5	0.43 –0.70 (0.62)	0.038–0.058 (0.050)	−16 to −35 (−22) 1 F
20	Sodium chloride	0.9%	" "	4	5	0.34 –0.65 (0.45)	0.022–0.050 (0.038)	0 to +14 (+4)
21	" "	5%	" "	4	4	0.40 –0.45 (0.43)	0.019–0.037 (0.029)	−60 to −88 (−77) 1 F
22	" "	10%	" "	4	6	0.23 –0.70 (0.44)	0.023–0.058 (0.043)	−34 to −90 (−60)

Values in parentheses are averages.
[1] Significant decrease in 1 instance.
[2] Significant decrease in 3 instances.
[3] Significant decrease in 2 instances.
'F' indicates ventricular fibrillation.

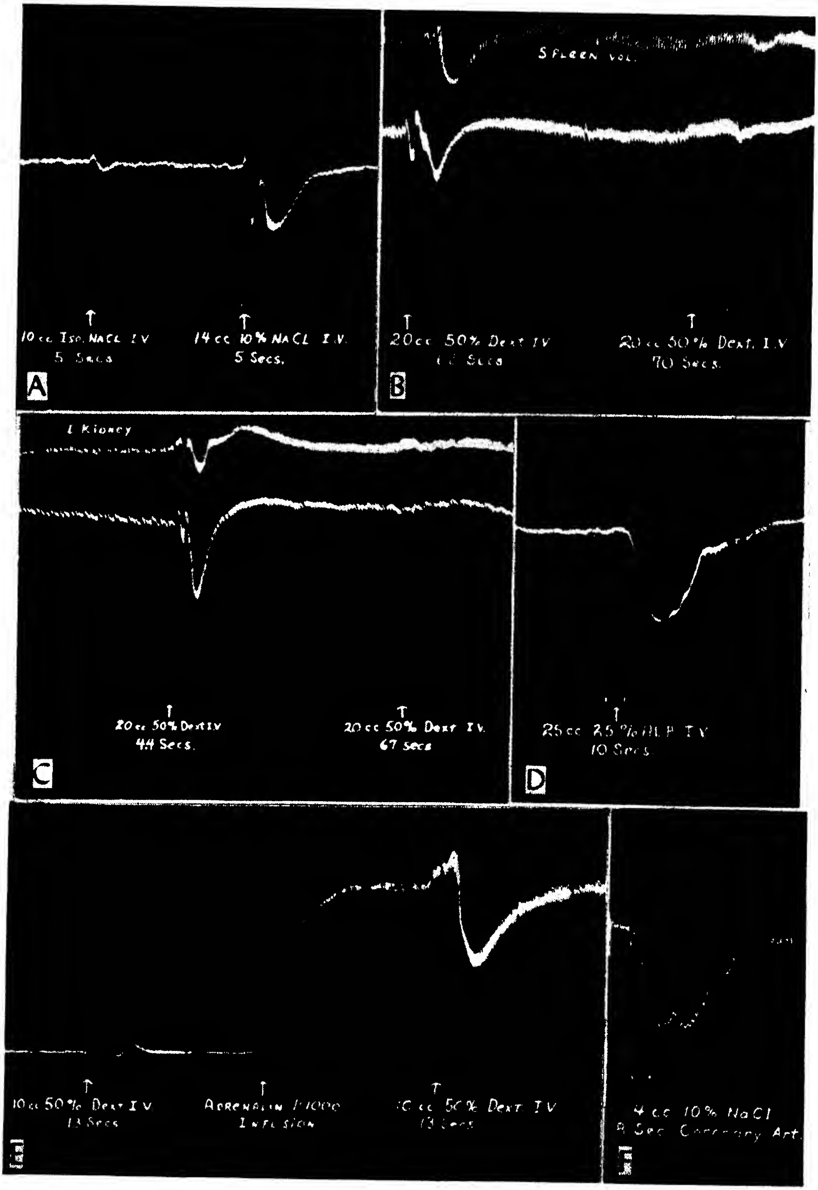

FIG. 1. EFFECT ON ARTERIAL BLOOD PRESSURE AND ORGAN VOLUME of intravascular injections of isotonic and hypertonic solutions.

6–8, table 1). The greatest drop was 16 mm. mercury. Usually, there was a slight increase in blood pressure during such slow injections (second injection of B of fig. 1).

The above data establish the fact that rate of injection is an essential factor in producing the 'transient depressor effect.'

Evidence that rapid intravenous injections of isotonic solutions do not lower the blood pressure is given in line 1, table 1. The volumes of solution and injection rates were in general as great as with the hypertonic solutions but a significant drop in blood pressure was encountered following only one of 23 injections. Not infrequently the injection of isotonic solutions produced a brief rise in blood pressure followed by a short decrease below the starting level (first injection of A of fig. 1). These fluctuations were greater with larger volumes and were entirely different in character from the type of blood pressure change elicited by the injection of hypertonic solutions. The slight transient drop observed appeared to be the result of overshooting of compensatory reactions called forth by the initial rise.

The blood pressure changes resulting from rapid injections of hypertonic solutions usually followed a characteristic pattern as shown in A, B, and C of figure 1. A slight rise at the start usually occurred, but this was followed by a depression which was much greater than the initial increase. The depression was much longer than with isotonic solutions and was usually diphasic. The first phase lasted 15 to 20 seconds and was more variable than the second phase. The second drop was nearly always greater and each time it lasted longer (45–60 seconds) than the first. The second rise was the most variable of the curve components and at times was entirely absent as exemplified in D of figure 1.

Since a characteristic and consistent drop in blood pressure resulted when hypertonic solutions were injected rapidly, it seemed desirable to devise experiments which would indicate the mechanism of its production. Two possibilities were considered: one, a sudden vasodilatation and two, a decreased cardiac output.

Oncometer tracings measuring kidney, spleen and intestinal loop volume changes were made when hypertonic solutions were rapidly injected into the femoral vein, carotid blood pressure changes being recorded simultaneously. On 40 occasions 10 to 15 ml. of 50% glucose were injected in 4 to 6 seconds into the femoral vein and in each instance the depressor effect was noted and at the same time the oncometer tracing registered a decreased organ volume (B and C of fig. 1). Thus, the visceral volume underwent passive changes that conformed as to direction with the changes in arterial pressure. On 12 occasions, similar volumes of isotonic solutions were injected rapidly (4–6 seconds) into the femoral vein. Negligible to no changes were observed in both blood pressure and oncometer tracings.

Venous pressure measurements were made in one femoral vein while rapid injections of hypertonic solutions were given in the other femoral vein. A composite curve of 8 such series of measurements is given in figure 2. A rise in the femoral venous pressure began around 30 seconds after the injection, reached its maximum at about 55 seconds and gradually returned to the pre-injection level after 3 to 4 minutes. At times the return to normal was much quicker. The average peak elevation of venous pressure above control values was 7.8 to 11.8 cms. H_2O. When these changes were compared with the arterial pressure

changes (depressor effect) a correlation was obtained. The elevation in venous pressure became prominent some seconds after the major depressor change in arterial pressure occurred.

The measurements of organ volume and venous pressure changes pointed to a decreased cardiac output rather than to vasodilatation as the cause of the transient depressor effect following rapid injections of hypertonic solutions by vein. However, none of the above experiments indicated whether this was a direct action on the cardiac muscle or a reflex phenomenon.

Electrocardiographic records revealed that no appreciable changes in heart rate occurred during the reaction. In fact, no consistent changes of any kind were seen in the electrocardiograms. On occasions there was a decrease in the

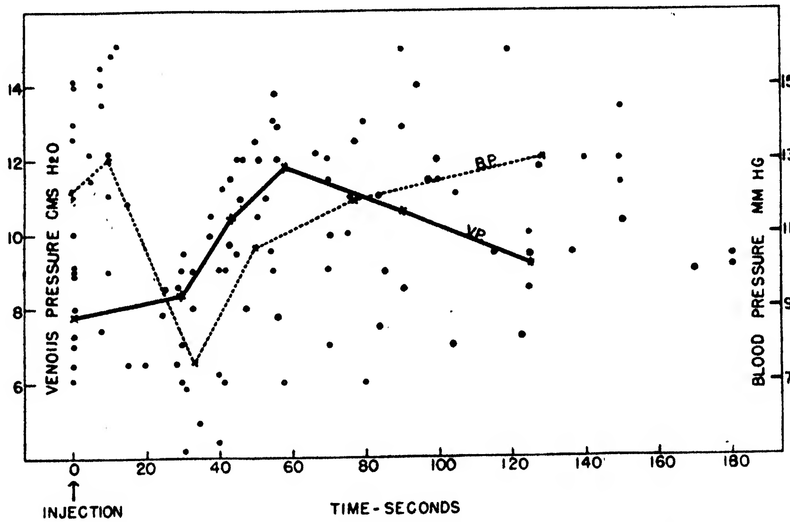

FIG. 2. RELATION OF VENOUS AND ARTERIAL BLOOD PRESSURE CHANGES following rapid injections of hypertonic solutions into the femoral vein.

amplitude of the R wave and in a few instances there was a transient inversion of the T wave. There also occurred occasionally an elevation of the S–T segment. The absence of marked changes in heart rate indicated that the effect was directly on the myocardium rather than through a reflex mechanism since in cardiac reflexes the chronotropic factor is commonly more pronounced than the inotropic. To evaluate any neurogenic elements in the reaction, the vagi were sectioned in 5 dogs and hypertonic solutions were injected rapidly into the femoral vein. The depression of arterial pressure occurred as in the intact animal. The same was true of another group of dogs which were atropinized. Since the action of the heart may be influenced by nervous mechanisms not involving the vagi, a group of dogs were pithed (destruction of brain and spinal cord) and then given rapid injections of 50% glucose into a femoral vein. Fol-

lowing the pithing procedure, artificial respiration was instituted and thus the animals could be maintained in a steady state for hours. Pithing resulted in a steady but very low mean arterial blood pressure. At this low blood pressure level, rapid intravenous injections of 50% glucose produced only a slight depression in the arterial blood pressure (line 2, table 2). This same diminution in depressive response was observed in dogs in which hypotension had been produced by hemorrhage (line 4, table 2). When a solution of adrenaline was slowly infused into a femoral vein of pithed dogs, a relatively normal blood pressure level could be maintained, and when 50% glucose was then injected rapidly the drop in blood pressure averaged 50 mm. Hg and the characteristic curve was traced (E of fig. 1 and line 3, table 2). Thus the depressor effect under these conditions equaled in magnitude that occurring in the anesthetized intact animal.

TABLE 2. *Relation of the hypotensive effect of hypertonic solution to the pre-injection blood pressure*

NUMBER OF ANIMALS	SOLUTION INJECTED	SITE OF INJECTION	CONDITION AND PRE-INJECTION MEAN ARTERIAL PRESSURE	VOLUME OF INJECTION	RATE OF INJECTION	CHANGE IN MEAN ARTERIAL PRESSURE
				ml/kg.	*ml/kg./sec.*	
5	50% glucose	Femoral vein	Normal 124–175 (157)[1]	1.0–1.7 (1.42)[1]	0.16–0.37 (0.27)[1]	−33 to −64 (−48)[1]
5	50% glucose	Femoral vein	Pithed 43–64 (52)	1.40–1.74 (1.59)	0.300–0.414 (0.335)	0 to −14 (−8)
5	50% glucose	Femoral vein	Pithed plus epinephrine 122–164 (140)	1.22–1.80 (1.53)	0.102–0.414 (0.267)	−28 to −70 (−50)
5	50% glucose	Femoral vein	Hemorrhaged 70–84 (78)	1.07–2.05 (1.68)	0.119–0.332 (0.258)	−10 to −36 (−28)

[1] Values in parentheses are averages.

It then became evident that the transient depressor effect was not neurogenic in origin but resulted from a direct response of the heart to hypertonic solutions. Therefore, injections at any part of the cardiovascular system permitting the undiluted solution to reach the heart should result in a drop in the blood pressure. To test this assumption, rapid injections of hypertonic and isotonic solutions were made into the left ventricle, right ventricle, root of aorta, abdominal aorta and femoral artery. Lines 9 to 15 of table 1 show the results. Rapid injections of hypertonic solutions into the heart chambers and the root of the aorta produced the usual depressor effect; injections into the abdominal aorta or femoral artery elicited no drop in blood pressure but in most instances resulted in a rise. Rapid injections of isotonic solutions produced no drop in blood pressure regardless of the site of the injection.

Since solutions injected into the root of the aorta could not directly enter the chambers of the heart but could enter the coronary circulation, hypertonic solutions entering the coronary circulation appeared to be the common factor explaining results obtained by injections at various sites. A procedure was

developed whereby injections could be made directly into the interventricular branch of the left coronary artery very near its origin from the main artery. Injections were made against the blood stream. Here, injection rates of less than 0.04 ml. per kgm. per second of hypertonic solutions regularly elicited the transient hypotension. This was true with volumes as low as 0.4 ml. per kgm. of 30% glucose or 5% sodium chloride, and with still smaller injections of more concentrated solutions. Control injections of isotonic solutions had no effect or resulted in a brief increase in blood pressure. Results of injections directly into the coronary circulation may be seen in lines 16 to 22 in table 1. Unlike injections into the femoral vein, these injections resulted in a blood pressure curve showing only a single depression; in no instance was it diphasic. The lack of diphasic configuration was also noted following injections into the left ventricle and root of the aorta. Injections into the right ventricle, on the other hand, produced curves such as previously described for femoral vein injections. A typical blood pressure response to rapid injection of a hypertonic solution into the coronary artery may be seen in F of figure 1.

DISCUSSION

In our search of the literature, a dearth of references dealing specifically with the effect of the intravenous administration of hypertonic solutions on blood pressure has been encountered. Retzlaff (3) in studying the efficacy of hypertonic salt solutions in restoring blood pressure after hemorrhage reported a transient further drop in the already low arterial pressure. In one instance, an injection of 3 ml. of 10% sodium chloride in the cat resulted in a monophasic drop in blood pressure similar to those we have encountered. Bernshtein (4) found that the intravenous injection of 10% sodium chloride or 50% glucose in the dog resulted in a temporary fall in blood pressure accompanied by an increase in heart rate. The fall in blood pressure was attributed to vasodilatation.

Hamm and Pilcher (5) in their experiments on cerebral blood flow observed that with the intravenous injection of 30% sodium chloride, a transient fall in arterial pressure resulted which may have been similar to the effects we have described. Apparently no significance was attributed to this initial effect and no attempt was made to determine the mechanism involved.

The hypotensive reaction we have observed following intravenous injections of hypertonic solutions is quite unlike the reactions described by Hanzlik and Karsner (6) and designated as 'anaphylactoid'. In our experiments the reaction is elicited by substances entirely physiologic in nature except for concentration. In some of our dogs given human plasma and human serum intravenously, we have observed on the first injection reactions similar to those described by Hanzlik and Karsner but this type of hypotensive reaction develops much more slowly, the recovery period is much longer and identical responses result whether the injection is made rapidly or slowly. After recovery from the 'anaphylactoid reaction', the animal is refractory to a second injection. However, at this time a rapid injection of any strongly hypertonic solution results in a typical hypotensive response.

Hirshfeld, Hyman and Wanger (7) concluded that the rapid intravenous injection of any molecular species results in widespread disturbances to which the term 'speed shock' was applied. The claim is made that concentration of the solution is not a factor in the production of this phenomenon, and that rapid injection of small volumes of isotonic solutions is effective in its elicitation. In our experiments with dogs anesthetized with pentobarbital sodium, we have found the rate of injection of small volumes of pharmacologically inactive substances to be entirely without significance. We do not believe that the intravenous injection of 1 to 5 ml. of such solutions as 0.1% sodium chloride or 0.9% sodium chloride ever produces a shock-like state in a dog no matter how rapid the injection, because in repeated control experiments with 0.9% saline and 5% glucose solutions using volumes larger than those employed by Hirshfeld and co-workers and injecting at faster rates, we have never observed so-called 'speed shock'.

We are convinced that the phenomenon which we describe is due entirely to the osmotic effect of the solution used and that the only significance of the rate of injection is related to the concentration attained in the coronary circulation. Our observations indicate that the rapidly injected volume of solution travels as a concentrated mass through the chambers of the heart and the pulmonary circulation and reaches the coronary vessels without sufficient dilution from admixture with blood or from fluid uptake in the lungs to interfere with the phenomenon. That the coronary circulation is the site of action is indicated by the fact that the hypotensive response ensues only when the concentrated solution is introduced at some point from which the coronary circulation is reached without traversing a set of systemic capillaries.

An interesting observation is that the hypotensive response is commonly diphasic when the hypertonic solutions flow through the chambers of the heart and monophasic when injection is made into the root of the aorta or directly into a coronary artery. In the latter instances, only the second or main depression of arterial pressure is elicited. It is conceivable that the less constant first depression results from a direct action on the myocardium while the solutions are within the chambers of the heart. The injection of strongly hypertonic solutions into the coronary artery produced a readily observable constriction of the vessels which quickly disappeared at the termination of the injection. The decreased blood supply to the myocardium may be a factor in the depressor response but the direct effect on the myocardium is, we believe, the more significant.

Concentrated protein solutions have been given intravenously to patients for various clinical reasons. On occasions when these solutions have been given at rates of 50 ml. in 30 to 60 seconds, a transient increase in pulmonary ventilation has been noted (8). Prominent hypotensive changes have not been observed but could have been missed since we have not used continuous recordings of blood pressure in man. Inasmuch as augmented respiration occurs during the hypotensive reaction in the dog and presumably as a consequence of the decreased

arterial pressure, it may be that the respiratory changes observed clinically are indicative of similar circulatory changes.

SUMMARY

Rapid intravenous injections of hypertonic solutions in dogs produce a marked transient drop in arterial blood pressure. This does not occur if hypertonic solutions are injected more slowly or if isotonic solutions are given rapidly. Oncometer recordings of organ volumes indicate that the hypotension is not due to vasodilatation. Venous pressure studies point to a decreased cardiac output as the causative factor. It is further found that rapid injections of hypertonic solutions into the right or left ventricles or the root of the aorta result in a sudden lowering of arterial blood pressure but the same injections into the abdominal aorta or femoral artery do not. This transient depression of blood pressure occurs in the vagotonized or atropinized dog or even when the brain and spinal cord are destroyed by pithing. Finally, this hypotensive effect is reproduced by injections directly into the coronary arteries but only if hypertonic solutions are used.

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EFFECTS OF CHRONIC STARVATION AND RECOVERY ON THE BLOOD OF THE YOUNG RAT

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Received for publication September 16, 1947

Numerous reports have appeared concerning the effects of chronic starvation on the blood of adult animals (1–7). Miller, Friedman and Deuel (8) have noted the influence of caloric restriction on the blood components of the young rat, and Keys *et al.* (9) have investigated rehabilitation from semistarvation in adult men. There are, however, no data in the literature on the effects of re-alimentation and recovery from chronic starvation in young growing animals.

Methods. 70 young male albino rats, 30 days old and of about 50 grams weight, were placed in individual cages and chronically starved for 12 weeks by restriction to a qualitatively adequate ration, adjusted daily to maintain a constant body weight. Measurements were made on the blood of 10 rats at the end of the underfeeding period. The remainder were refed for an additional 8 weeks and were studied at the close of this time. Determinations on the numbers of red and white cells and on the specific gravity of the whole blood were made on blood taken from the tip of the tail. The specific gravity of the plasma, the hemoglobin, hematocrit, non-protein nitrogen, plasma protein and blood sugar were determined on blood which was drawn from the heart with a 'heparinized' syringe after anesthesia by nembutal. In addition to the experimental animals, similar terminal data were collected on two control groups of 10 rats each; one was the same weight as the starved rats and the other was the same age as the starved rats. Progressive changes in the total red and white cells and in the specific gravity of the blood during starvation and recovery were determined on a separate group of rats by taking blood from the tails at 7-day intervals. Parallel progressive studies were made on 10 young normal rats of the same initial weight as those which were to be starved.

The hematocrits were determined by centrifuging for one hour at 2500 R.P.M. with the blood in a Wintrobe tube. Total white and red cell counts were determined by employing standard dilution pipettes, diluting fluids and hemocytometers. Hemoglobin was determined by the Sahli method. The specific gravity of the whole blood and of the plasma were determined by the copper sulfate method of Phillips *et al.* (10). Plasma protein values were calculated by use of the nomogram of these authors. Blood N.P.N. was determined by nesslerization after digestion with an acid digestion mixture. Blood sugar was determined by the Folin-Wu method. All colorimetric determinations in connection with the above were made with a Klett colorimeter.

Results. *The effects of chronic starvation on the blood.* Terminal data are summarized in table 1. The plasma protein, hemoglobin and hematocrit values of the young underfed rats were higher than in normal rats of the same weight

and lower than in normal rats of the same age, indicating that these features of the blood continued to increase with age during caloric restriction, although at a reduced rate. These results may be at variance with those of Miller, Friedman and Deuel (8) who considered the blood of underfed rats similar to that of normal rats of the same weight and interpreted the blood values as a function of size or maturation.

The fact that in the experimental animals the red cell count and specific gravity of the blood obtained from the tail were nearly normal, while the hematocrit, plasma protein and hemoglobin of the blood obtained from the heart were decreased, may indicate that semistarvation in young rats produces a redistribution of the blood effecting a relative peripheral hemoconcentration. This result is interpreted as a compensation mechanism. The marked increase in the red cell count and the specific gravity of the blood during early starvation as shown

TABLE 1. *Effects of chronic starvation and recovery on the hematocrit, red and white cell numbers, specific gravity, non-protein nitrogen, plasma protein, hemoglobin, and sugar of the blood of the young rat*

EXPER. GROUP	HEMATOCRIT	[1]SPECIFIC GRAVITY OF BLOOD	NON-PROTEIN NITROGEN	PLASMA PROTEIN	BLOOD SUGAR	HEMOGLOBIN	[1]RED CELL NUMBER	[1]WHITE CELL NUMBER
	per cent		*(mgm./100 cc.)*	*(gram/100 cc.)*	*(mgm./100 cc.)*	*per cent*	*(/cu. mm.)*	*(/cu. mm.)*
Normal (30 days)	33.0	1.0471	—	3.77	—	64.0	5,873,000	9,855
Starved	41.0	1.0610	75.7	4.85	103.3	78.3	8,557,000	5,405
Refed	45.0	1.0594	38.4	5.40	105.0	89.8	8,609,000	14,000
Normal (6 months)	47.0	1.0600	43.2	5.48	119.5	92.0	8,920,000	20,130

[1] Determinations made on blood from tip of tail.

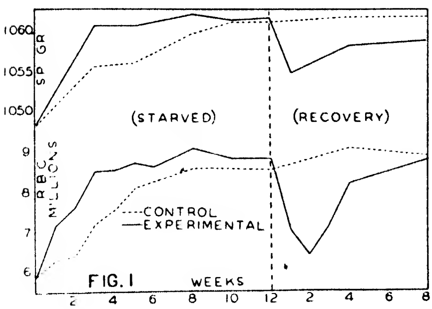
in figure 1, may be further evidence of this circulatory adaptation, the effectiveness of which waned as underfeeding was prolonged.

At the end of the starvation period there was only a slight difference between the specific gravity of the blood of the underfed rats and the blood of normal rats of the same age. This result is in agreement with Benedict (2), who states that there is no material variation in the density of the blood as a result of starvation, and with Lust (11), who found that the blood in chronic malnutrition changes but slightly in water content. Observations of a hydremia, however, have been reported by Jackson (1) and Henschel *et al.* (12). In this experiment one might have expected such a hydremia with the relatively increased water intake which was observed in the starved rats. Since the density of the blood did not change, it must be that there was a retention of water by tissues other than the blood or an increased loss of water for metabolic and excretory purposes.

Figure 2 reveals that a gradual reduction in total white cells appeared during starvation. This result is in agreement with related investigations made by Ershoff and Adams (5), Wright and Skeggs (6) and Keys *et al.* (7). Only Minot (13) found the white count unaltered after prolonged underfeeding. No measure-

ments were made in this study which explains the mechanism of this leucocyte reduction. Morgulis (4) states that the quickness of the onset of leucopenia shows that it cannot be due to a failure of new cells entering the blood stream but to migration of leucocytes into the intestinal wall and lumen. However, as presented in figure 2, no such sudden leucopenia occurred in the young semistarved rats of this experiment. Consequently, if the suddenness of leucocyte decrease in inanition constitutes the proof of leucocyte migration, then this investigation does not necessarily corroborate such an interpretation.

It would be expected that acute starvation would increase the blood N.P.N. because of the ultimate catabolism of body proteins as a source of energy. There remained the question, however, of the effect of semistarvation in which enough food was provided to meet all energy demands except growth. The high N.P.N. in the underfed rats of this study could not have been due exclusively to the



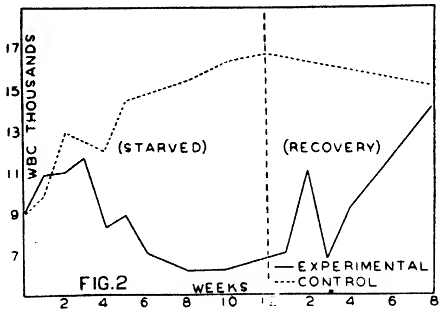


FIG. 1. Red cell number and specific gravity of the blood during 12 weeks' chronic starvation and 8 weeks' recovery.

FIG. 2. Alteration in the total leucocyte count during 12 weeks' chronic starvation and 8 weeks' recovery.

metabolism of body proteins since enough carbohydrates and fats were permitted in the daily ration to prevent progressive weight loss by utilization of body tissues for energy. It appears necessary then to look elsewhere for an explanation. Schaffer and Lee (14) state that the N.P.N. is decreased by the growth hormone and conclude that the latter is a stimulant of protein anabolism. Teel and Cushing (15) and Teel and Watkins (16) find a similar reduction in N.P.N. as a result of injection of an extract of anterior pituitary. Braier (17) reports a decrease in nitrogen excretion after hypophysectomy. It is clear from the work of these men that the pituitary gland and its growth hormone have a profound effect on nitrogen metabolism. Since, as Mulinos and Pomerantz (18) have shown, chronic malnutrition creates a condition of pseudo-hypophysectomy, it may be that the high N.P.N. in the underfed rats of this experiment is directly explicable as a result of a functional alteration of the pituitary. The only other feasible cause for such a high N.P.N. would be a relatively small fluid in comparison to the nitrogen to be excreted, as mentioned by Bray (19). It is un-

likely that this explanation is valid, however, since as already noted the animals during starvation used large amounts of water in proportion to food intake.

No significant alteration in the blood sugar appeared in the starved rats of this investigation. This result is in contrast with a similar type of starvation conducted on adult human males by Keys *et al.* (8) in which a progressive fall in blood sugar was observed. The age maturity difference of these human subjects as contrasted with the young immature rats used in this experiment probably accounts for the difference in results.

The effects of refeeding on the blood. Table 1 shows that the recovery of the plasma protein, hemoglobin, total leucocyte and hematocrit values was nearly complete after 8 weeks refeeding. At the end of this recovery period the red cell count, specific gravity of the blood and the blood sugar remained within the normal range. The blood N.P.N. was also restored to a normal value, indicating that the phase of pituitary function associated with protein metabolism had fully recovered.

Figure 1 shows a marked reduction in specific gravity of the blood during early refeeding. Comparison with figure 2 reveals that there was a concomitant leucocytosis. This recovery hydremia was transient and was probably due to the relatively rapid regeneration of the plasma. A similar hydremia during refeeding has been mentioned by both Rosenstern (20) and Jackson (1).

SUMMARY

The plasma protein, hemoglobin and hematocrit of young semistarved rats were higher than in normal rats of the same weight and lower than in normal rats of the same age. These values continued to increase with age during caloric restriction, but did so at a rate which was less than that in fully fed growing rats.

During underfeeding the peripheral blood of the starved rats had a greater density and red cell count than the blood of normal rats of the same weight, but this difference became less marked with time so that at the end of the starvation period the values were nearly the same. This relatively high density and red cell count of the peripheral blood, together with the reduced hematocrit, hemoglobin and plasma protein found in blood drawn from the heart, may indicate that chronic starvation in young rats produced a redistribution of the blood so that there was a relative peripheral hemoconcentration.

Chronic starvation did not significantly alter the blood sugar, but there was a marked increase in the N.P.N. and a gradual decrease in the white cell number.

A hydremia and leucocytosis occurred almost immediately upon realimentation as shown by the decrease in the red cell count and the specific gravity. This effect was temporary and in early recovery the density and white cell count of the blood were rapidly returning to normal.

At the end of the refeeding period the blood had recovered to the extent that it presented a normal or nearly normal picture in all respects studied.

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REDUCED CARBOHYDRATE INTAKE AFTER FAT FEEDING IN NORMAL RATS AND RATS WITH HYPOTHALAMIC HYPERPHAGIA[1]

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Received for publication October 2, 1947

It has been known for at least a century that starvation or low carbohydrate intake causes certain abnormalities in the carbohydrate metabolism of animals and human beings. Claude Bernard (4) noted glycosuria in dogs given food after a period of starvation. It is now well known that, following a period of low carbohydrate intake, the glucose tolerance curve is higher and longer than normal ('starvation diabetes', 2, 3, 11, 18). Only the previous carbohydrate intake, not the fat, protein or total caloric intake, determines the form of the glucose tolerance curve (10).

The blood sugar curve after insulin injection is likewise dependent upon the previous diet, being steeper after a high carbohydrate intake (1, 10, 19).

An abnormally low rise of the respiratory quotient after glucose administration has been found in animals after prolonged starvation or a low carbohydrate intake (9). After a short period of low carbohydrate diet the RQ response has been found normal (12) and after a moderately low carbohydrate intake (20–25% of the calories) the RQ curves after glucose have been found even higher than normal, in spite of the fact that the blood sugar curves showed the usual starvation diabetes pattern (13).

It has also been shown that fasting animals, which have previously been kept on a low carbohydrate diet, deplete their carbohydrate stores more slowly and survive longer than animals kept on a high carbohydrate diet (17). Finally it has been found that the rise of blood pyruvate after glucose administration is abnormally sluggish in persons who have fasted for 6 days (20).

The exact cause of these abnormalities is not known. The insulin content of the pancreas is low on a fat diet (5), but the abnormal blood sugar curves after insulin injection show that this cannot be the sole determining factor. The difference between fasting animals previously fed carbohydrate or fat persists after hypophysectomy (16).

The above results have been obtained by observing what happens when carbohydrate is administered after a period of starvation. No studies seem to have been performed on the spontaneous food intake on change from a low to a high carbohydrate diet.

Investigation of hypothalamic obesity has brought into the foreground the

[1] These experiments were aided by a grant from the Life Insurance Medical Research Fund to Drs. C. N. H. Long and John R. Brobeck.

[2] University of Copenhagen Fellow and James Hudson Brown Memorial Fellow.

problem of regulation of food intake (6, 8). From these studies it is seen that the primary, if not the only demonstrable disturbance in these animals after hypothalamic lesions, is a greatly increased food intake. It was, therefore, thought of interest to study on different diets the spontaneous food intake of normal animals and animals with hypothalamic lesions, and especially the effect of changing the animals from a period of carbohydrate starvation to a diet rich in carbohydrate.

METHODS AND DIETS

Plateaued female rats of the Sprague-Dawley strain have been used throughout. During the experiments the animals were kept in a constant temperature room at 86°F. They were allowed to eat the different diets ad libitum. The food intake was determined each day by weighing the food cups, and the animals were weighed three times a week. In the experiments with changing diets the animals were kept in metabolic cages for the collection of urine. Methods for blood sugar and urinary glucose were the same as described in earlier papers from this laboratory (6). The hypothalamic lesions were made with the Horsley-Clarke instrument as described earlier (6).

The diets had the following composition:

CARBOHYDRATE DIET		FAT DIET	
	grams		*grams*
Sucrose	710	Sucrose	0
Lard	0	Lard	315
Casein	200	Casein	200
Corn oil	50	Corn oil	50
Osborne-Mendel salt mixture	40	Osborne-Mendel salt mixture	40
	1000		605

To the diets was added a vitamin mixture which supplied the following amounts of synthetic vitamins to 100 grams of the carbohydrate or 60.9 grams of the fat diet:

	mgm.		*mgm.*
Thiamine	0.20	Biotin	0.02
Riboflavine	0.30	Folic acid	0.05
Pyridoxine	0.25	2-Methyl-naphthoquinone	0.10
Nicotinic acid	2.00	Inositol	10.00
Pantothenic acid	2.00	Choline	100.00

In addition, 2 mgm. of α-tocopherol in halibut liver oil were given to the animals weekly.

A difficulty was encountered with the casein used for the preparation of the diets. In the beginning commercial casein, obtained from Lister Bros., New

York, was used. Later this brand became unavailable and another kind had to be used, 'Casein B3F' from Casein Co. of America, New York. Hereafter these two caseins will be referred to as casein *A* and *B*.

The substitution of the new brand of casein in the diets altered the food intake, presumably because of a different content of certain micronutrients (strepogenin ?).

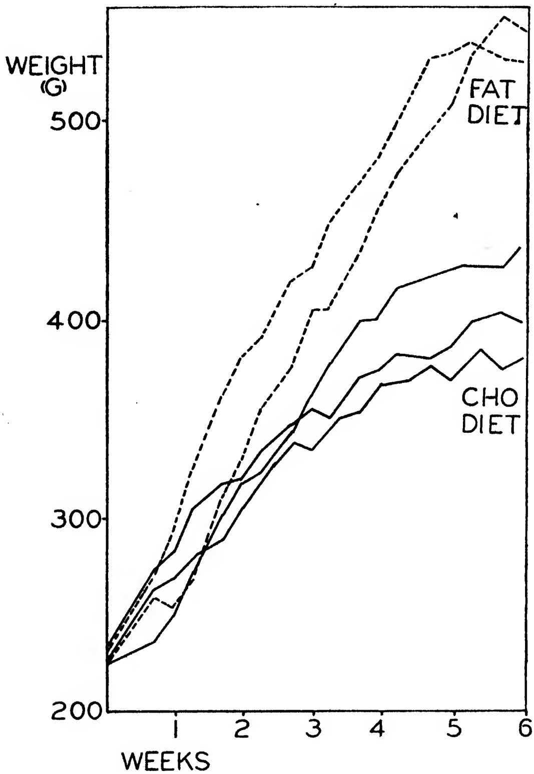

FIG. 1. WEIGHT CURVES OF RATS fed carbohydrate or fat diet. Hypothalamic lesions were made at 0 weeks.

As will be seen, however, this disadvantage did not materially influence the principal results of the investigation.

RESULTS

Two kinds of experiment were performed. (*a*) Normal animals and animals with hypothalamic lesions were kept for a long period on either the carbohydrate or the fat diet. (*b*) Normal animals and animals with hypothalamic lesions were fed a carbohydrate and a fat diet alternately for short periods of 1–2 weeks. Casein *A* was used for the diets in the 3 first groups of experiments and casein *B* in the last one.

Normal animals kept for 16 weeks on either a carbohydrate or fat diet. The food intake was uniform throughout this period for the 6 animals studied. The average caloric intake was 39 Calories per day on the carbohydrate diet, and 41 Calories per day on the fat diet. In spite of this the animals on the fat diet gained on the average 0.6 gram per day, while the animals on the carbohydrate diet kept their weight practically unchanged.

After 16 weeks casein *B* was substituted in the diets. Following this change the food intake of the animals on the fat diet increased about 10%, while no increase was seen on the carbohydrate diet.

Animals with hypothalamic lesions on either carbohydrate or fat diets (fig. 1). The food intake was higher immediately after the operation on the fat diet and stayed high. On the carbohydrate diet the intake increased in the course of the first week to a level which was lower than the level of the fat-fed animals and then fell off after 3 weeks. The animals on the fat diet gained weight more rapidly and for a longer time than the animals on the carbohydrate diet. The weight gain per calorie per day was significantly higher on the fat diet than on the carbohydrate diet, 0.066 gram/Cal/day as against 0.048 gram/Cal/day ($t = 3.6$; $t_{0.05} = 2.3$).

Animals with hypothalamic lesions on changing diets (fig. 2). Of 4 animals, 3 were started on the fat diet, one on the carbohydrate diet.

The animals started on the fat diet showed a higher caloric intake and a steeper weight curve on the fat diet than on the subsequent carbohydrate diet. After the second fat period, when the weight of the animals had increased greatly, the food intake on the carbohydrate diet was further reduced. In the two fattest animals it was negligible. The animal which was started on the carbohydrate diet did not show any significant change of caloric intake on change to the fat diet, while the weight curve increased in steepness. But after the subsequent shift to carbohydrate diet a pronounced fall in food intake and weight occurred.

Normal animals on changing diets (Table 1). In this experiment casein *B* was used for the diets of the 6 animals.

In the first period the food intake was higher on the fat diet than on the carbohydrate diet, just as in the last part of the first group of experiments where the same kind of casein was used.

The pattern obtained by changing the diet was, however, essentially the same as in the obese animals on changing diets, except that no extra depression of carbohydrate intake occurred at the second change to this diet. The animals started on the fat diet showed a fall of caloric intake and weight on change to the carbohydrate diet. The animals started on the carbohydrate diet showed no change of food intake on changing to the fat diet but an increase in weight. Upon subsequent change to the carbohydrate diet a fall in caloric intake was seen as in the group started on the fat diet. After the first feeding period the food intake on the two diets was remarkably regular: on the carbohydrate diet, 27.2–28.0–32.0 Calories; on the fat diet, 51.3–48.7–51.3 Calories.

Certain of the animals showed in a few of the periods a tendency to 'adapta-

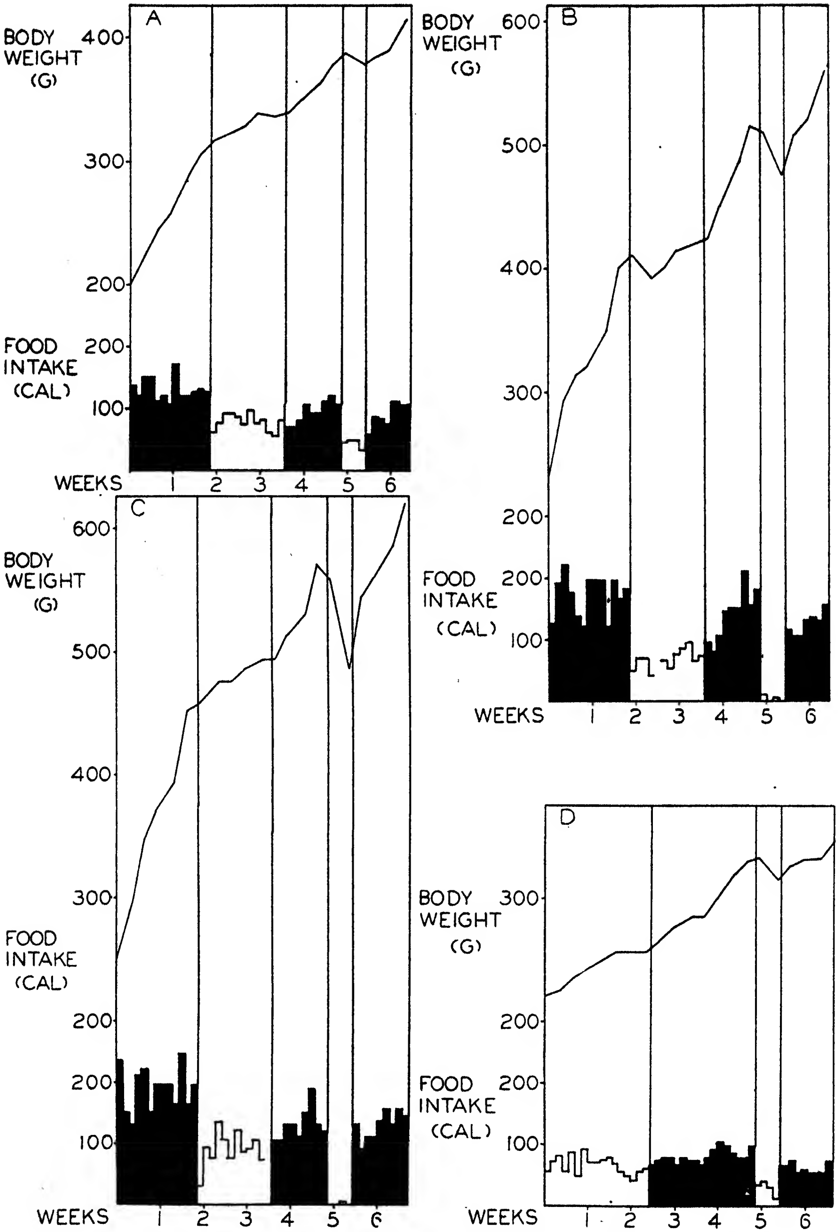

FIG. 2. WEIGHT AND FOOD INTAKE CURVES of 4 rats with hypothalamic lesions during consecutive periods on fat or carbohydrate diet.
(Solid columns—fat diet; open column—carbohydrate diet.)

tion' to the diets, i.e., a gradual fall of caloric intake after some days on a fat diet or a rise after some time on the carbohydrate diet, respectively.

Blood sugar and urinary glucose. The blood sugar of the obese animals on the two diets was followed every 4 hours for 24 hours. No significant difference was observed. On the carbohydrate diet all the animals, obese as well as normal, showed a slight glycosuria varying from traces to 0.2–0.4%. These small losses of glucose persisted throughout the carbohydrate feeding period, and are therefore probably not comparable to the short glycosuria of hunger diabetes in dog or man.

TABLE 1. *Average values for caloric intake and weight change of normal rats during consecutive periods of 2–3 weeks on fat or carbohydrate diet*

	PERIOD 1 FAT DIET	PERIOD 2 C.H. DIET	PERIOD 3 FAT DIET	PERIOD 4 C.H. DIET	
Cal/day........	61.3	27.2	51.3	28.0	
Weight change, grams/day...	+1.8	−1.1	+1.6	−0.8	
		PERIOD 1 C.H. DIET	PERIOD 2 FAT DIET	PERIOD 3 C.H. DIET	PERIOD 4 FAT DIET
Cal/day........		48.9	48.7	32.0	51.3
Weight change, grams/day...		+0.6	+1.2	−0.6	+1.4

DISCUSSION

The principal finding in the experiments has been that the caloric intake is unchanged, both in normal and obese animals, on a change from a high carbohydrate to a high fat, noncarbohydrate diet, while a considerable decrease is seen in both groups of animals on the reverse kind of change. This seems to indicate that the phenomenon is not simply one of different food intakes on different diets. The sequence of events is important: a period of high fat intake, or carbohydrate starvation, reduces the animal's appetite for carbohydrate.

It seems natural to try to relate the decreased food intake observed in our experiments to the above-mentioned abnormalities of carbohydrate metabolism. These abnormalities can be stated broadly by saying that normal animals have a certain limited ability to dispose metabolically of ingested carbohydrate. A reduction of this ability or capacity is found in hunger diabetes, i.e., after a period of starvation or carbohydrate undernutrition. Our results show that normal animals respond to this reduction by a decrease of food intake when carbohydrate diet is given after a period of high fat, carbohydrate-free diet.

Hypothalamic lesions cause an increase of food intake on any diet. When the animals are fed a high carbohydrate diet they eat less than when they are fed a fat diet, as if their food intake were limited by their capacity to dispose of carbohydrate. If the animals are allowed to eat a fat diet after the operation

they eat more, and if they are thereafter changed to a carbohydrate diet, a fall in caloric intake occurs.

It is difficult to reconcile what is known about the food intake of diabetic animals with the above findings. On the one hand we know that pancreatectomized animals have a high food intake on a high carbohydrate diet; on the other hand it has been shown in self-selection experiments that diabetic animals choose less carbohydrate than normal animals, thereby keeping free of glycosuria and other diabetic symptoms (15).

In the experiments of Brobeck, Tepperman and Long (7) in which hypothalamic lesions were made in partially pancreatectomized, nondiabetic animals, a severe glycosuria developed with the onset of the high food intake. The primary reduction of the capacity to dispose of carbohydrate, obtained by the partial pancreatectomy, seems to have made it impossible for these animals with augmented appetite to keep below their 'limit of disposition'.

The other result of our study, that the weight gain per calorie is greater on the fat diet than on the carbohydrate diet, is probably due to the fact that the animals on the fat diet do not have the cost of a transformation of carbohydrate to fat. If the specific dynamic action of the two diets used here were determined, a somewhat higher rise would be expected by giving the carbohydrate than the fat diet. The demonstration of a greater weight gain, as compared with that on isocaloric intake of carbohydrate, might perhaps be of importance in view of a certain prevailing tendency to oversimplification of the relation between food intake and obesity.

Finally, the finding of a greater depression of food intake by change from fat to carbohydrate in the animals with hypothalamic lesions, and especially the extreme reduction seen after the development of gross obesity, points to the fact that the obese state in itself is a determinant of the capacity of the carbohydrate-disposing mechanisms. This is in accordance with the slightly abnormal glucose tolerance curves found in obese animals by Brobeck, Tepperman and Long (7). The carbohydrate disposing mechanisms seem to be fitted to a certain body size. When the weight of the animal is greatly increased after a period of hyperphagia, the caloric intake on a high carbohydrate diet falls off more rapidly than on a high fat diet, and the reaction to dietary change from fat to carbohydrate is excessive.

The special type of diabetes melitus in the obese, described by Newburg and Conn (14), which disappears after weight reduction, is probably related to this phenomenon. In human beings the appetite regulation is influenced by many more factors (habit, environment, psychological structures, etc.) than in animals. Human diabetics—in contradistinction to rats—do not select a beneficial low carbohydrate diet. Obese human beings on the verge of glycosuria do not curb their appetite and may develop a diabetic state.

SUMMARY

Following the production of hypothalamic lesions the food intake and weight gain of rats are greater on a high fat, noncarbohydrate diet than on a high carbohydrate diet.

Normal animals, as well as animals with hypothalamic lesions, show a decrease in food intake when changed from a fat diet to a carbohydrate diet. This reduction is greater in obese animals.

The relation between this regulation of food intake and the abnormalities of carbohydrate metabolism in hunger diabetes, as known from earlier studies of blood sugar, respiratory quotient and blood pyruvic acid, is discussed.

We wish to express our gratitude to Drs. C. N. H. Long and John R. Brobeck for their interest and valuable suggestions during the performance of this work as well as to Dr. W. A. Krehl for his advice on the composition of the diets.

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INCREASED TOLERANCE TO SEVERE ANOXIA ON CARBON DIOXIDE ADMINISTRATION[1]

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Received for publication September 16, 1947

Under ordinary conditions of living, various types and degrees of oxygen deprivation are commonly experienced. War in its modern form greatly augments the incidence of anoxic conditions; it was thus to be expected that the recent emergency would provoke a greatly expanded program of investigation of such disturbances, especially those met with in aerial activities at high altitudes. The experiments described herein represent the outgrowth of a program initiated in 1941, the principal findings of which were described in a recent publication (1). Circulatory conditions particularly at low barometric pressures and the effects of various oxygen, carbon dioxide and nitrogen concentrations are now considered.

METHODS

Lightly narcotized (sodium amytal) and heparinized cats were used throughout the experiments described. The 'altitude chamber' was a modified autoclave with a capacity of 127 liters; ventilation was carried out at a rate of 14.5 l/min. at S.T.P. and was increased in inverse relation to decreased pressure during evacuation. Chamber temperatures were kept between 20° and 25°, the level in any group of experiments usually varying no more than 1°. Simulated altitudes were taken from the data given by Armstrong (2). E.C.G. records (left 5th interspace, left ear leads), E.E.G. (occipital cortex, left ear leads), and waves of respiratory activity (abdominal musculature lead) were obtained by means of a Grass electroencephalograph, properly filtered. Arterial pressure readings were secured by cannulation of the carotid artery, the T-cannula being directly connected to a mercury manometer. The rate of simulated ascent of 4000 ft/min. (maximum ceiling, 50,000 ft.; 87 mm.Hg bar. pressure) was duplicated, it should be emphasized, in all experiments. Administration of gas mixtures was accomplished by ventilation of the chamber with the desired gases at a sensitively controlled rate of 14.5 l/min. S.T.P.

Experimental criteria. In order to compare the responses to anoxia and various gas mixtures, certain criteria derived through experience were adopted as indicative of respiratory and cardiovascular failure: *a*) E.E.G.: complete disappearance of 'cerebro-cortical' potentials; *b*) respiration: pronounced slowing of the respiratory rate; *c*) arterial pressure: rapid fall in systolic and diastolic pressures, with marked increase in pulse pressure; and *d*) heart: pronounced bradycardia;

[1] The experiments described were undertaken as part of a program of research performed under contract (N6 ori-116) with the United States Navy, Office of Naval Research.

abnormalities of the T-wave, such as increase in amplitude and/or inversion; partial block.

With the appearance of the above phenomena, the animal was found to have reached a critical stage of anoxia; it was thus considered the 'critical level' or 'breaking point', beyond which continuation of the experiment would lead quickly to death. In practically all experiments and always in cases in which

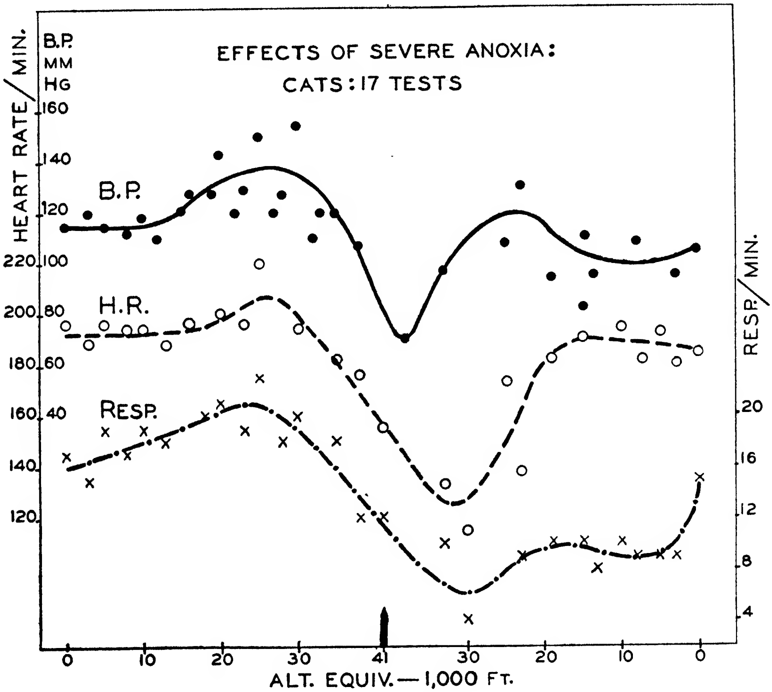

FIG. 1. EFFECTS OF EXTREME ANOXIA on arterial pressure, heart and respiratory rates, as shown in 17 experiments on 10 cats. Under the conditions imposed, the critical level was observed at an altitude equivalent of *c.* 41,000 feet.

repetitive tests were conducted with one animal, rapid descent to sea-level conditions was begun immediately after this 'breaking point' was reached. Thus, control and experimental runs were possible with each animal.

Control experiments. Responses in control tests to severe anoxia are shown in figure 1. The curves presented give average figures obtained from 17 exposures of 10 cats. The initial response to the progressive anoxia imposed was invariably an increase in both arterial pressure and heart rate, as well as in frequency and/or depth of respiration, at a (average) simulated altitude of 12,000 feet, or 483 mm.Hg. Striking responses to lower altitudes did not appear, although individual variations were sometimes present.

Parallel increases in arterial pressure and heart and respiratory rate continued

until a simulated altitude of 28,000 feet (247 mm.Hg) was reached, when all three criteria showed sharp declines in value. This was most marked in the case of arterial pressure. Cardiovascular and respiratory failure was now progressive, and at 41,000 feet (134 mm.Hg) profound depression in blood pressure, coupled with the appearance of gross abnormalities in E.C.G. waves, indicated that the critical level had been reached.

With descent, arterial pressure increased rather abruptly; the heart rate, however, continued to fall until a simulated altitude of 34,000 feet (187 mm.Hg) was

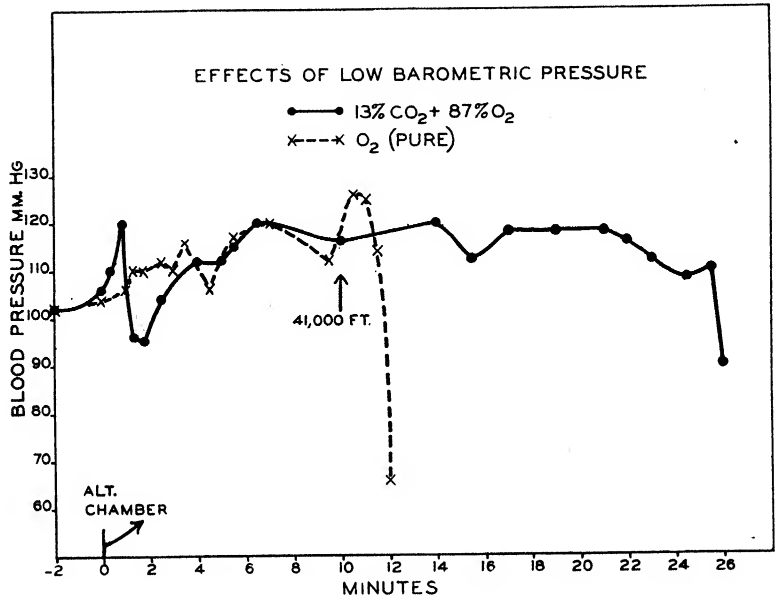

FIG. 2. EFFECTS ON BLOOD PRESSURE following the inhalation of pure oxygen in comparison with an oxygen-carbon dioxide (87%—13%) mixture. In both instances the animal (cat) was subjected to 134 mm. Hg (alt. eq. 41,000 ft.) and kept at that level. Note maintenance of normal blood pressure for 15 minutes by the experimental animal as compared with 2 minutes only for the control.

reached, when an increased rate first became evident. At 15,000 feet (429 mm. Hg), both arterial pressure and heart rate approached normal, but respiratory activity was not fully restored until the barometric pressure approached that at sea level.

That a simulated altitude of 41,000 feet represented approximately the critical level under the experimental conditions set up was repeatedly shown. There were many individual variations, however, and each animal served as its own control in all experiments undertaken.

Effects of carbon dioxide on altitude tolerance. To determine whether an increase in altitude tolerance might be effected by the addition of carbon dioxide

to the respired gases, experiments were conducted in which the chamber was continuously ventilated with O_2 or O_2-N_2 mixtures; for control purposes, and comparison made with changes on exposure to O_2-CO_2 and O_2-CO_2-N_2 mixtures. In all tests, the critical level was established in control runs, although the reverse order of exposure (i.e. experimental, control) was sometimes followed. On return to sea-level conditions, an animal was allowed a rest period (at normal barometric pressure) for 20 to 30 minutes.

Oxygen—carbon dioxide (87%—13%). As shown in figure 2, a definite increase in altitude tolerance resulted on the addition of carbon dioxide to the respired oxygen. Thus, the critical level for oxygen was found to be 41,000 feet, collapse occurring within a very brief period at this altitude.

The short period of tolerance at 41,000 feet in the presence of pure oxygen does not necessarily mean that the cat is supersensitive as compared to human

TABLE 1. *A comparison of tolerance times to circulatory collapse under severe anoxia*
Continuous ventilation with *a*) O_2 and *b*) O_2-CO_2 (87%-13%)

CAT NO.	BAROMETRIC PRESSURE	ALT. EQUIV.	TOLERANCE TIME TO COLLAPSE	
			(a) Under O_2	(b) Under O_2-CO_2
	mm. Hg.	*ft.*	*min.*	*min.*
1	134	41,000	2	14
2	122	43,000	1	3
2	122	43,000	0.5	4
3	122	43,000	0	3
4	134	41,000	2.5	10
5	134	41,000	1.5	6
Averages			1.3	6.7

subjects. It is possibly due, as pointed out by Oster and Smith (3), to the depressant action of barbital compounds on the central nervous system.

On allowing carbon dioxide (13%) into the system with oxygen, cardiovascular failure did not occur at the same altitude until 14 minutes had elapsed. That is, the addition of carbon dioxide enabled the animal to tolerate an altitude of 41,000 feet for a period of 14 minutes longer than when oxygen alone was respired. This increase in tolerance is further illustrated in table 1, which shows that the presence of CO_2 in the respired gas increases the resistance to anoxia many-fold, considered on the time basis.

Oxygen-nitrogen-carbon dioxide (20%-67%-13%). Before studying further the effects of carbon dioxide under the conditions of continuous ventilation, reactions under a control mixture of 80% nitrogen and 20% oxygen were established. Responses to this mixture did not vary from those observed during ventilation with air. The critical level was found to be 41,000 feet. After a rest period the animals were exposed to a gas mixture containing 20% oxygen, 67% nitrogen and 13% carbon dioxide, and the chamber again evacuated to the simulated (critical) altitude of 41,000 feet. Results of these experiments may

be evaluated by reference to figure 3, representing an average of findings from 16 experimental and 14 control tests on 3 animals.

It will be noted that with the addition of carbon dioxide to the gas mixtures, the arterial pressure rose an average of 19 mm.Hg above the normal, while in animals exposed to the oxygen-nitrogen mixture alone, a definite fall in arterial pressure occurred. Although a decrease in heart rate was seen in all cats

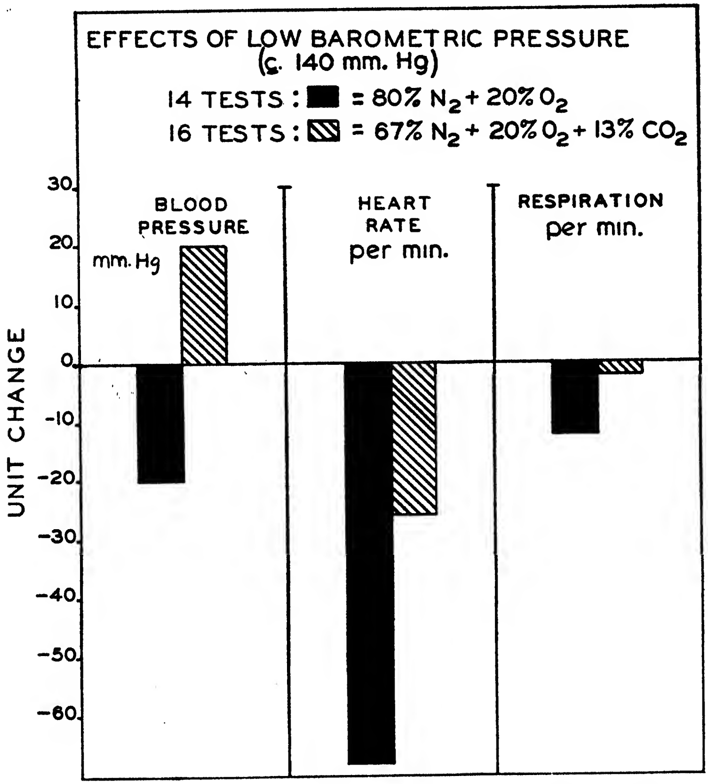

FIG. 3. BLOOD PRESSURE, HEART AND RESPIRATORY RATES in animals breathing an oxygen-nitrogen (20%—80%) mixture, compared to oxygen-nitrogen-carbon dioxide (20%—67%—13%). Graph represents changes at 'breaking point' established with O_2–N_2 (20%—80%).

employed in these experiments, it amounted to only 25 beats per minute in those exposed to the carbon-dioxide mixture, compared to an average slowing of 61 per minute in the control tests. Further, while the respiratory rate was reduced by 12 per minute when employing the O_2-N_2 mixture, it was well maintained following the addition of carbon dioxide. Specific data also are given in table 2.

Pre-exposure to oxygen. It appeared desirable to compare experiments in which animals were pre-exposed to O_2 and O_2-CO_2 mixtures both at normal barometric pressure and under a positive pressure of two atmospheres, thus saturating

the blood with oxygen as completely as possible before exposing the animals to anoxic conditions. Each animal was subjected to a control exposure in air to determine its critical level of response. When this value had been ascertained, the animal was returned to sea-level conditions, where a 'rest period' of from 20 to 30 minutes was permitted prior to the experimental run.

A marked increase in tolerance was noted in experiments in which the cats were pre-exposed to oxygen. Normal or elevated blood pressures and heart rates were maintained at altitudes from 11,000 to 13,000 feet higher than in control tests (fig. 4). It may be observed that varying the time of pre-exposure,

TABLE 2. *Circulatory protection by CO_2 in anoxia*

Series I., O_2-N_2 (20%–80%); Series II., O_2-N_2-CO_2 (20%–67%–13%). (Administered continuously during exposure)

CAT NO.	BAROMETRIC PRESSURE	TEST NO.	I. O_2-N_2		II. O_2-N_2-CO_2	
			Arterial pressure	Heart rate	Arterial pressure	Heart rate
	mm. Hg.		*mm. Hg*	*min.*	*mm. Hg.*	*min.*
6	148	1	70	156	88	171
		2	66	150	90	179
		3	64	149	78	165
		4	64	138	78	156
		5	68	137	98	156
		6	68	140	96	162
		7	67	145	106	148
7	128	1	74	126	130	142
		2	70	113	114	128
		3	61	102	106	148
		4	80	102	120	133
		5	81	119	134	130
		6	78	115	144	125
Experimental Average.............			70	133	106	149
Average at beginning of tests (I and II)......................			92	194	87	175

within the time limits employed (10 to 40 min.), did not alter tolerance to any significant degree.

Cats pre-exposed to oxygen under a pressure of two atmospheres showed a further slight increase in tolerance compared to those animals pre-exposed to oxygen at 760 mm.Hg for a like period.

Pre-exposure to oxygen-carbon dioxide (87%—13%). That the addition of carbon dioxide to the respired gases in pre-exposure tests increased the tolerance to anoxia is illustrated in figure 5. In this case, the animal was pre-exposed for 10 minutes to a mixture of 87% oxygen and 13% carbon dioxide at normal barometric pressure. Tolerance was now shown at a simulated altitude 9,000 feet higher than under control (air) conditions and 2,000 feet higher than when pre-exposed to oxygen alone for a similar period of time. Moreover, normal arterial

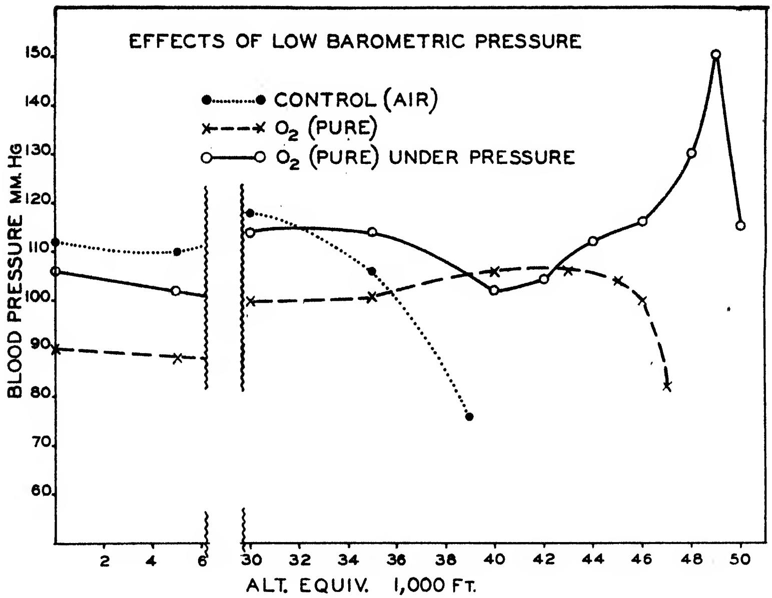

FIG. 4. EFFECTS ON CAROTID PRESSURE of severe anoxia; influence of previous exposure to pure O_2. The symbols refer to pre-exposure conditions.

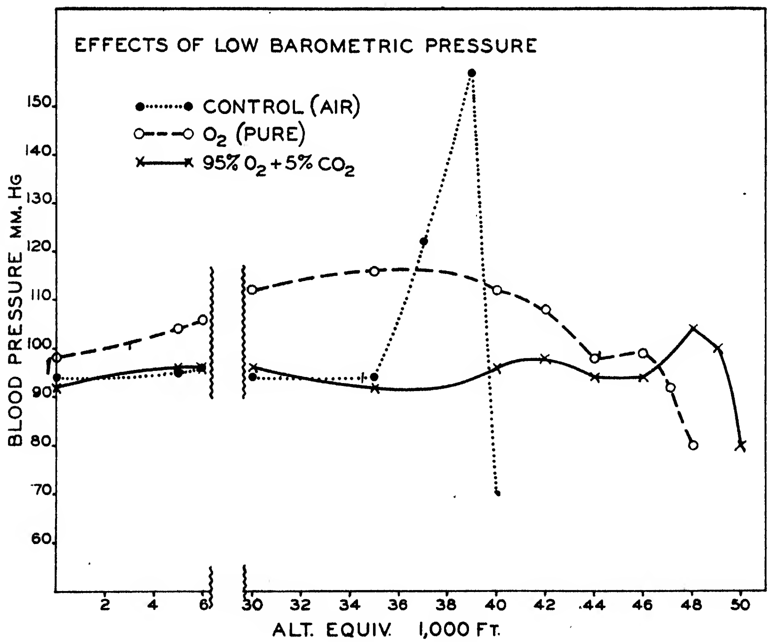

FIG. 5. EFFECTS OF PRE-EXPOSURE (10 min.) to *a*) pure oxygen and *b*) an oxygen-carbon dioxide (95%—5%) mixture on arterial (carotid) pressure under severe anoxia. Symbols refer to pre-exposure conditions.

pressure and heart and respiratory rates were well maintained at the critical level as established after pre-exposure to oxygen alone.

Resuscitation at the 'breaking point'. Further experiments were performed to compare the restorative power of oxygen alone and an oxygen-carbon dioxide (87%—13%) mixture. Animals were carried in these cases to an altitude equivalent of 33,000 feet and maintained there until the 'breaking point' was reached. At this time, either O_2 or the O_2-CO_2 mixture was used to replace the air in ventilating the chamber. It was observed that normal arterial pressure and heart and respiratory rates were quickly restored in both cases. Except for a more rapid recovery of the normal respiratory rate following the use of the O_2-CO_2 mixture, there appeared to be very little difference in the restorative effect of the two respired gases in this type of experiment.

DISCUSSION

The criteria used in the present study as indications of immediately impending cardiovascular and respiratory collapse due to anoxia have been described by numerous observers as indications of extreme oxygen deficiency. Thus, the precipitate fall in arterial pressure, with increased pulse pressure, has long been known to be characteristic of the anoxic state (4, 7). That bradycardia is the inevitable result of oxygen deprivation has been reported by almost all students of the subject (4, 6–9) and variations in T-wave characteristics have also been frequently described (4, 5, 10, 11). The observation that heart block appears in the latter stages of anoxia has likewise been reported (4, 12).

The selection of that degree of anoxia at which all of these signs appear concurrently, and its designation as the 'critical level' or 'breaking point', was made on the basis of its utility in the present investigation; it was found most helpful in the comparison and evaluation of results obtained with the various gas mixtures employed. Obviously the time and intensity of an exposure must be considered in all such experiments.

That the addition of carbon dioxide to the gases respired at 'altitude' may have a beneficial effect in counteracting the effects of oxygen deficiency has previously been reported (13, 14), but its *modus operandi* has remained the subject of much debate. Gellhorn (13) has advanced the opinion that the beneficial effects of carbon dioxide derive from peripheral vasoconstriction and concomitant cerebral vasodilatation which follow its inhalation—a view with which the findings of Lennox and Gibbs (15) are in agreement. The above investigators also point to increased pulmonary ventilation and oxyhemoglobin dissociation within the tissues as possible explanations for the observed beneficial effects. Gibbs *et al.* (14) point out, moreover, that the maintenance of carbon-dioxide tension within the brain tissues may be equal in importance to the maintenance of oxygen tension. The foregoing results on electroencephalographic and respiratory studies may be interpreted as supporting these contentions.

The present observations and also some by other investigators indicate that carbon dioxide, inhaled in optimum concentration, is of considerable aid in increasing tolerance to anoxia. Yet opinion on this point is not unanimous; Dill

and Zamcheck (16) have concluded, for example, that pure oxygen is of more benefit than oxygen-carbon dioxide mixtures, on the basis of studies on oxygen saturation in the blood.

The present barometric pressure chamber study suggests strongly that under the influence of carbon-dioxide inhalation, both time at 'altitude' as well as 'ceiling' under conditions of uniform rate of ascent would be considerably increased. The increase of ceiling on CO_2 administration by 2000 to 3000 feet over that attained by oxygen inhalation, at the high altitude equivalents of 40,000 to 50,000 feet, appears highly significant.

SUMMARY

Mild anoxia in the cat (483 mm.Hg or 12,000 ft. equiv. alt.) was characterized by increased heart rate and arterial pressure, as well as frequency and depth of respiration.

The anoxic 'critical level' or 'breaking point' was reached, under the standard rate of decompression used, at 134 mm.Hg barometric pressure or an altitude equivalent of 41,000 feet. Ordinary exposures at this 'altitude' for a matter of seconds (30 to 90) resulted in severe collapse and sometimes death unless immediate restorative measures were instituted.

The breaking point was characterized by *a*) marked slowing of both the respiratory and heart rates, *b*) a sudden fall in systolic and diastolic arterial (carotid) pressures with increased pulse pressure, *c*) abnormalities in form of the T-wave in the electrocardiogram and *d*) various degrees of heart block. These cardiovascular and respiratory changes were coincident with *e*) the disappearance of occipito-cortical potentials as shown in the electroencephalogram.

Addition of carbon dioxide (13%) to physiologically appropriate oxygen-nitrogen mixtures increased altitude tolerance significantly. This was evidenced by increased survival time at a set altitude, as well as by increase in ceiling attained before the onset of cardiovascular and respiratory collapse.

The author gratefully acknowledges much help and encouragement from Dr. S. W. Britton in carrying out this investigation.

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TYPES OF AFFERENT FIBERS IN THE PHRENIC NERVE

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Received for publication October 8, 1947

It is well established that the phrenic nerve contains afferent fibers which affect the activity of the respiratory center. The fact that reflexes affecting the cardiovascular system may also be elicited is not generally appreciated. The existence of such phrenic afferents was indicated by the casual observations of Dingle *et al.* (1) that stimulation of the central end of the cut phrenic nerve sometimes elevated mean arterial pressure and increased the total peripheral resistance.

Subsequent search through the literature revealed that a pressor response to phrenic nerve stimulation had been reported as early as 1868 by Kowaleski and Adamük (2). This was confirmed by Kowaleski and Nawrocki (3) and Henoque and Eloy (4). In 1883 Schreiber (5) stimulated the dorsal roots and thoracic portion of the phrenic nerve with electrical and mechanical stimuli and noted that the increases in blood pressure varied with the type and intensity of stimulus. He observed no changes in the frequency and intensity of the heart beat. In 1884, v. Anrep and Cybulski (6) excited the central cut end of the phrenic nerve in narcotized and curarized animals. They noted a series of wave-like oscillations in arterial pressure which they believed to be Traube-Hering waves. Malschin (7) in 1922 obtained increases in pressure in one series of experiments but none in another. He also observed no changes in heart rate but noted acceleration and deepening of respiration with blood pressure changes. In 1930 Mussgnug (8) performed three experiments on dogs noting blood pressure increases in two animals and a decrease in the third. In 1935 Greene (9) stimulated the central cut end of the phrenic nerve and found changes in blood pressure, heart rate, and coronary flow. He stated that animals varied greatly and negative results were often obtained.

In his extensive review of vasomotor reflexes McDowall (10) mentioned the phrenic nerve only to say that Grossman obtained negative results from afferent stimulation. With the exception of Greene (9), Dingle *et al.* (1) and Mussgnug (8), the previously mentioned workers recorded blood pressures by means of mercury manometers and counted heart rates visually or by palpation. They are, we believe, subject to the criticism of McDowall (10) that most investigators have not excluded cardiac effects on blood pressure.

METHODS

Experiments were performed on 20 mongrel dogs weighing from 10–20 kilos. They were anesthetized with morphine sulfate subcutaneously and sodium bar-

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bital administered intravenously. The thorax was opened by a midsternal incision and artificial respiration was given. The phrenic nerves were cut low in the thorax and the central ends stimulated with platinum electrodes encased in glass shields as described by Sherrington. These served as moist chambers. Stimuli were produced either by a Harvard inductorium or by electronic stimulators. It was found early in the work that simultaneous stimulation of the two phrenic nerves gave better results, hence both nerves were stimulated simultaneously. Blood pressure in the arch of the aorta was recorded from the left subclavian artery by means of a calibrated Gregg manometer of adequate sensitivity and frequency. Artificial respiration was carefully adjusted so that spontaneous movements of the thorax and abdomen were just absent or minimal. Records of spontaneous movements which resulted during stimulation were obtained by transmitting the motions of the left half of the split sternum to a receiving capsule connected to a Frank segment capsule. In some experiments right atrial pressures were measured and kidney volumes recorded by an air oncometer also connected to a Frank segment capsule.

RESULTS

Effects on respiratory movements. Since artificial ventilation of the lungs remained constant in our experiments any change in spontaneous respiratory movements were due to direct reflex effects. In eight animals the effects were similar to those shown in figure 1. In this record inspiration is downward. Slight natural movements were present during control periods. Immediately after phrenic excitation respiratory tonus decreased while the inspiration became deeper and more prolonged. As a rule the respiratory rate also increased but this was not invariable. The effects gradually wore off after cessation of stimulation and the smaller control movements were resumed within 20–30 seconds. In a number of experiments the movements during control periods reciprocated with a rhythm of lung inflation (Hering-Breuer reflexes). In these cases stimulation of afferent phrenic fibers increased the magnitude of the natural movements but did not affect the rhythm. In four experiments illustrated by figure 2 the natural respiratory movements were very slight and phrenic excitation also had no appreciable effect. The changes in vagotomized animals are shown in figure 3 in which inspiration is recorded as an upward movement. In such experiments stimulation of phrenic afferents still caused a greater amplitude and duration of inspiration but no change in expiratory tonus. The effects gradually diminished during the half minute following cessation of stimulation

The changes in respiration interested us chiefly with regard to the effects on arterial pressure, hence no attempt was made to integrate the reflex changes with other reflex drives of the respiratory center.

Effects on heart rate. Three types of responses were observed after stimulation of phrenic afferents; *a*) absence of any change in heart rate; *b*) an increase in heart rate synchronous with the rise of arterial pressure; and *c*) cardiac slowing at or near the summit of the pressure rise.

Six of our animals developed no initial rise in heart rate upon stimulation of

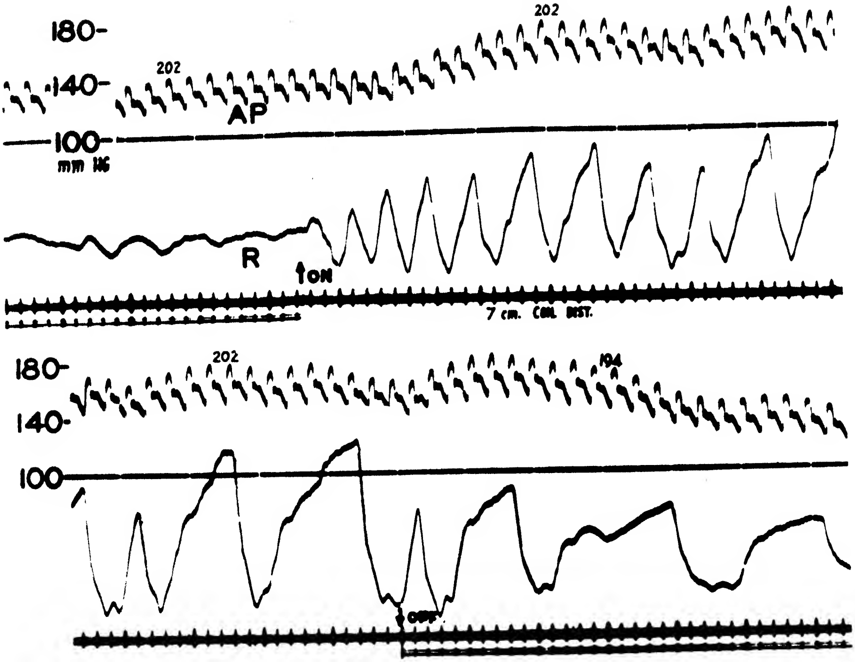

FIG. 1. UPPER AND LOWER RECORDS of this figure show continuous effects of stimulating the central end of phrenic nerves. Upper record, aortic pressure (AP); lower record, natural respirations (R). Onset and offset of stimulation marked on record. Numerals over aortic pressure record indicate heart rates.

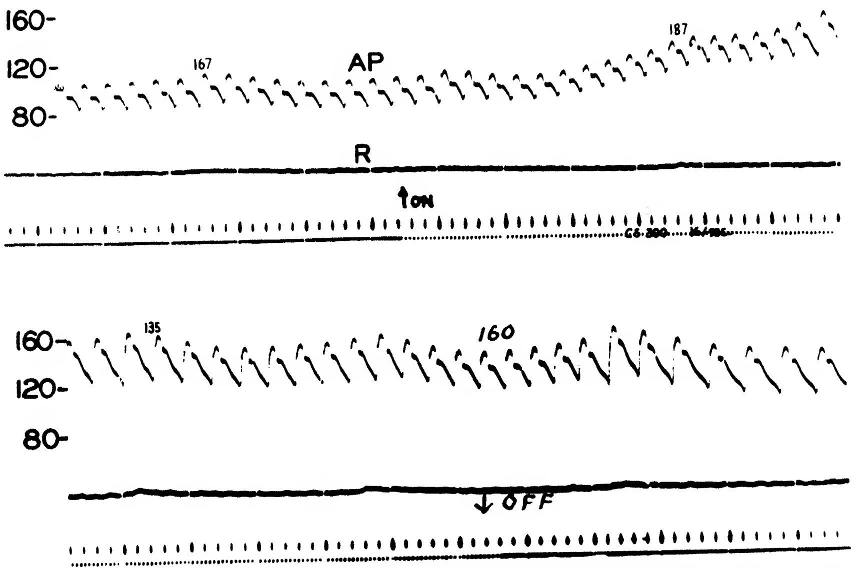

FIG. 2. EFFECT OF STIMULATING BOTH PHRENIC NERVES on aortic pressure (AP) and respiration (R). Other lettering as in figure 1.

the central ends of the phrenic nerves. Such an example is shown in figure 1. In five animals a significant increase in heart rate occurred as the pressure rose. However, as shown in figure 2, this was replaced by a progressive deceleration until the heart rate fell to or below control values. In other experiments it was demonstrated that this slowing was abolished by vagotomy as illustrated in figure 3. Previous to section of the vagus nerve in this experiment the heart rate was 137 and decreased to 125 per minute.

It is obvious that when changes in heart rate develop in response to stimulation these must be considered in relation to alterations in arterial pressure and vice versa. Thus in the experiment shown in figure 2 it may not be assumed without other evidence that the initial increase in arterial pressure is due to rise of peripheral resistance; it may have been due to the initial cardiac acceleration. So also the secondary slowing which occurs may not be due to direct synaptic connections between phrenic afferents and the vagus center; it may be occasioned as a secondary effect following the rise of arterial pressure. In this connection it is important to note that when pressure declined during continuance of phrenic excitation the heart accelerated again.

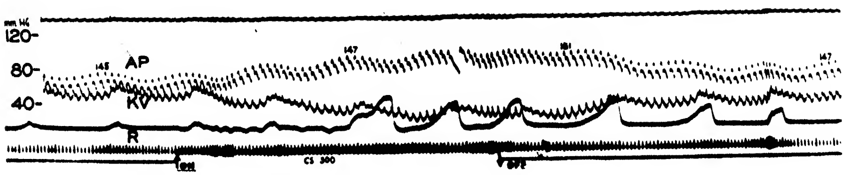

FIG. 3. EFFECTS OF STIMULATING CENTRAL END OF PHRENIC NERVES during period marked on record upon aortic pressure (AP), kidney volume (KV), and natural respiratory movements (R). Other markings as in figure 1.

Pressor and depressor effects. The changes in arterial pressure during stimulation of phrenic afferents consisted either of pressor or depressor responses in blood pressure. Figures 1 and 2 show typical pressor responses. In the former aortic pressure rose within five seconds from 135/117 mm. Hg to 185/157 mm. Hg. The rise was maintained throughout stimulation except for mechanical effects produced by periodic lung inflation. After cessation of stimulation the pressures gradually dropped to levels below those of the control period but eventually returned again to the previous level. This recovery effect is omitted in the figure to save space.

The changes in pulse pressure were variable, but as a rule an increase was shown, as in figure 1. Occasionally, however, it decreased as shown in figure 2. Depressor reactions were of two types; *a*) an immediate decline such as shown in figure 3 or *b*) a secondary decline following the initial increase as shown in figure 1. Analysis of all experiments revealed no correlation with changes in heart rate or respiration. Of the 10 animals which displayed a secondary depressor effect, respiratory movements were increased in five and unaltered in four. Cardiac slowing was always accompanied by a decline of arterial pressure, but the converse was not true.

According to Gordon (12) and Ashkenaz (13) the type of arterial pressure response following stimulation of afferent nerves depends on the frequency. Accordingly, the effects of changing the strength and frequency of shocks applied to the phrenic nerve were investigated. For this purpose electronic stimulators were used by which the strength and frequency of shocks could be varied independently. The two stimulators had time constants of 3 msec. and 0.3 msec., respectively. A study of results from six experiments revealed that regardless of stimulus strength, a frequency of 25 per second was the highest which elicited a depressor response. In most cases the best reductions in arterial pressure followed use of frequencies between 1 and 10 per second. Pressor responses on the other hand could be elicited with any frequency between 5 and 100 per second, provided an appropriate intensity of current was used.

In many experiments a reversal of a depressor to a pressor response could be effected by merely changing the frequency of stimulation. Thus in one experiment 34 volts applied at a rate of 2 per second yielded a depressor response. When the rate was changed to 3–11 per second a pressor response eventuated. Changes in frequency of stimulation were far more important than alterations in the strength of stimulus. The depressor thresholds ranged from 5–60 volts, whereas pressor thresholds extended almost through our voltage range, which was up to 250 volts. The effect of changing the intensity of stimulation was shown in one experiment, in which the frequency of stimulation (5 per second) and its duration were kept constant. With low strengths of current, pressor responses only were observed. As the intensity of stimulation increased a diphasic reaction, namely, a depressor followed by a pressor response, was observed. Further increase in intensity of stimulation resulted only in a pressor response. Such changes have been observed without alteration in heart rate.

Factors concerned in production of pressor and depressor responses. We have already emphasized that some of the changes in arterial pressure which follow stimulation of phrenic afferents are due to changes in heart rate. The possibility that increased respiratory movements of the diaphragm may augment venous return and thus increase systolic discharge is excluded in our experiments by section of both phrenic nerves. However, as shown by Lewis, Werle and Wiggers (11), contraction of the spleen following stimulation of afferent nerves can increase venous return and cardiac output considerably and thereby contribute to the elevation of arterial pressure. It therefore remained to establish the existence of direct reflex vasoconstriction in more definite ways. That such direct effects occur was strongly suggested in previous studies by Dingle *et al.* (1) who found that the calculated total peripheral resistance increases upon phrenic nerve stimulation. It is also supported by experiments in our series in which no alterations in heart rate were induced and no increase in right atrial pressure could be detected. In order to obtain still more conclusive evidence of peripheral vascular changes we recorded changes in kidney volume in four experiments. Figure 3 reveals that the kidney volume (KV) declines as the arterial pressure increases during stimulation of the central end of the phrenic nerve with a strong current of high frequency. On the basis of such information we may conclude

that the pressor responses obtained denote reflex vasoconstriction. With regard to depressor responses our results were not as conclusive.

DISCUSSION

Our results showed that afferent fibers of the phrenic nerve connect centrally with the respiratory, cardiac acceleratory and vasomotor centers, but not all of these effects are always elicited in every animal studied. The most constant reaction was on the respiratory center. Cardiac effects were less common than pressor or depressor responses. If connections exist with the cardio-inhibitor center, these must be in the nature of a depression, for the initial response consists of acceleration. The slowing which supervenes occurs at the summit of the pressor reaction and is abolished by vagus nerve section. It is more likely, therefore, that it is a secondary effect due to stimulation of the sinus caroticus nerves by the rise in pressure.

The fact that the accelerating effect persists after vagotomy strongly suggests either that direct connections are made with the cardio-accelerator centers or alternately with centers evoking increased discharge of epinephrine from the adrenal medulla. In either event connections must occur with sympathetic efferents.

The pressor and depressor effects obtained are in part accounted for by alterations in heart rate. Evidence presented indicates clearly, however, that it is due mainly to activation or depression of the vasomotor center. By selective stimulation using different frequencies and strengths of current, it could be shown that the pressure response attributable to vasomotor changes tends to be in the direction of a decline at frequencies below 25 per sec. and in the direction of a rise of pressure at higher frequencies. But at low frequencies the strength of the stimulus decidedly affects the direction of the vasomotor response; strong shocks cause pressor, weaker ones depressor effects.

SUMMARY

1. The central ends of the two phrenic nerves were excited by induction or condensor shocks. Arterial pressures, respiration and in some instances changes in kidney volume and right atrial pressure were inscribed by optically recording systems.

2. An analysis of results indicates that afferent fibers of the phrenic nerve communicate with respiratory, cardio-accelerator and vasomotor centers, activities of which result in a rise of arterial pressure. The slowing effects sometimes observed at the height of the pressure rise are abolished by vagotomy and are therefore due to secondary effects on the carotid sinus.

3. By selective stimulation with shocks of different strengths and frequency, it was found that the current strength as well as the frequency determines whether pressor or depressor reactions due to vasomotor action are obtained.

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EVIDENCE FROM CROSSTRANSFUSION EXPERIMENTS THAT THE DIMINISHED URINE FLOW ACCOMPANYING ISCHEMIC COMPRESSION SHOCK IS NOT DUE TO HUMORAL FACTORS[1]

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Received for publication October 6, 1947

It is a common observation that urine formation is depressed in various states in which either hypotension or decreased cardiac output occurs, such as shock, heart failure and Addisonian crises, as well as in toxemias of pregnancy. It has been suggested that this reduction in urine flow may be due *a*) to vasoconstriction (1) and that this might be due to a humoral substance (2, 3); *b*) to a substance that might possibly lead to excessive tubular reabsorption (4–6); and *c*) that the tubules might be blocked by precipitation within them of protein material (5–8). Another possibility which must be considered is that the lowering of mean arterial pressure and consequent diminution in renal blood flow reduces the glomerular filtration pressure below a critical level, at which no further glomerular urine is formed.

This paper is a report of a study of the severity of reduction of urine flow in ischemic compression shock in the dog and an analysis of the relative parts played by humoral factors and by the level of the mean arterial pressure. The former has been studied by measuring simultaneously the urine flow in traumatized dogs and in normal tests dogs crosstransfused with the traumatized dogs, and the latter by comparison of the urine flow in traumatized dogs with that in control dogs whose mean arterial pressure has declined to comparable levels without their being subjected to trauma.

METHODS

The procedures for inducing trauma and for crosstransfusion were essentially the same as those reported in previous papers (9–11). Ischemic compression shock was induced in 9 dogs (trauma dog, table 1, groups I and II) by wrapping both hind legs from the ankle to the groin with tightly pulled rubber tubes (9). Upon removal of the tubes, the arterial pressure of the traumatized dog rapidly fell, and the dogs died in shock within 3–9 hours despite blood and saline infusions given to 6 of them in large amounts.

In 5 experiments (table 1, group I) blood from such a traumatized dog was continuously exchanged with the blood of a normal test dog by way of a stromuhr

[1] Aided in part by a grant from the Ella Sachs Plotz Foundation.

[2] The authors wish to acknowledge the technical assistance of Misses Helen Hilderman and Nancy Kester, and Mr. William McCall.

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(10, 11). In each experiment, the test dog supplied blood from its femoral artery and received the traumatized dog's blood by way of its femoral veins. The traumatized dog supplied blood by way of its carotid artery in three experiments, and in two experiments by way of a cannula inserted into the distal end of the inferior vena cava. In all instances the blood from the test dog was received into the jugular vein of the traumatized dog. The use of the stromuhr assured that each dog received exactly as much blood as it gave. In each experiment the crosstransfusion was started about 45 minutes before the compression tubes were removed from the trauma dog. If a drop occurred in the mean arterial pressure of either dog the crosstransfusion was stopped until the arterial pressure had returned to the previous level, which required 15 to 45 minutes.

All dogs were anesthetized with an initial subcutaneous injection of 2 mgm/kgm. of morphine followed by sodium pentobarbital[4], 20 mgm/kgm. intravenously. Additional amounts of the latter were given as necessary to maintain anesthesia. The blood of both dogs was prevented from clotting by an initial intravenous injection of 0.25 ml/kgm. of heparin solution[5] followed by one-fifth this amount every half hour.

Zero time is taken as the moment of release of the compression tubes; in the control experiments it is 0.5 to 0.75 hours after the start of the crosstransfusion. The crosstransfusion was maintained for an average of 4.5 hours or until the prior death of the traumatized dog. The mean arterial pressures of the two dogs were recorded with mercury manometers beginning 1 to 2 hours before the start of the crosstransfusion and continuing until 1 to 2 hours after its completion. The cannulas were then removed from the surviving animals, the incisions sutured and the dogs placed in recovery cages.

Urine flow was measured by catheterizing the bladder. Both male and female dogs were used. The urine volume was measured at 15-minute intervals throughout the period during which mean arterial pressure was recorded.

In order to observe the effects of the crosstransfusion procedure per se upon urine flow, 6 normal dogs were crosstransfused (table 1, group III) and in 7 experiments normal dogs were autotransfused by exchanging their blood through the stromuhr, between the artery and vein of one hind leg and the artery and vein of the opposite leg (table 1, group IV). Urine flow was followed in 4 additional traumatized dogs used in a separate study (table 1, group II).

RESULTS

Urine flow in trauma dogs. In all but one of 9 dogs subjected to ischemic compression trauma (table 1, groups I and II), the urine flow dropped to very low levels during the first hour after release of the compression tubes and, in all but two, practically ceased after the first hour. The reduction in urine flow occurred despite the administration of large volumes of blood or saline. In these dogs the

[4] The sodium pentobarbital was kindly supplied by Premo Pharmaceutical Labs., Inc., New York, N. Y.

[5] The heparin was 'Liquaemin,' which was supplied through the courtesy of Roche-Organon, Inc., Nutley, New Jersey.

TABLE 1

EXP. NO.	ANIMAL	WT.	CROSS-TRANS-FUSION REACTION[a]	CESSATION OF URINE FLOW DURING REACTION[b]	CONTROL PERIOD[c]				FIRST HOUR				SUBSEQUENT HOURS				TIME WHEN URINE FLOW CEASED[d]	DURATION OF STUDY	LOWEST PRESSURE AT WHICH URINE FLOW CONTINUED[e]	MAP AT WHICH URINE FLOW CEASED	SURVIVAL[d]	VOL. EXCHANGED—LITERS—AFTER ZERO TIME
					Infusion		Av. urine flow	Av. MAP	Infusion		Av. urine flow	Av. MAP	Infusion		Av. urine flow during period when flow persisted	Av. MAP						
					Blood	Saline			Blood	Saline			Blood	Saline								
Group 1 Crosstransfusion traumatized dog with normal test dog																						
		kg.			ml/min.	ml/min.	ml/min.	mm. Hg	ml/min.	ml/min.	ml/min.	mm. Hg	ml/min.	ml/min.	ml/min.	mm. Hg	hrs.	hrs.	mm. Hg	mm. Hg	hrs.	
KH32[f]	Trauma	14.0	0	0	0	0	0.26	160	0	0	0.03	80	0	0	0.0	50	0.8	2.3	85	65	2.3	2.2
"	Test	12.8	0	0	0	0	0.17	105	0	0	0.10	90	0	0	0.24	120	—	5.5	70	—	surv.	2.2
A28	Trauma	14.0	0	0	0	0.61	0.19	150	1.11	0	0.04	70	1.30	0	0.19	90	—	4.5	70	—	5.6	21.0
"	Test	9.8	+	+	0	0	0.09	130	0	0	0.06	135	0	0	0.06	110	—	4.5	110	—	17.0	21.0
A29	Trauma	10.9	0	0	0	0.53	0.73	110	4.80	0.50	0.12	85	0.64	0	0.06	85	3.0	5.0	70	65	5.0	24.0
"	Test	9.6	+	0	0	0.42	0.04	100	0	0.35	0.04	100	0	0.41	0.06	130	—	5.0	100	—	19.3	24.0
A31	Trauma	14.2	0	0	0	0.38	0.15	95	5.55	1.00	0.06	75	0	1.00	0.0	60	1.0	4.5	75	65	4.5	25.8
"	Test	5.6	+	0	0	0.40	0.23	140	0	0.37	0.16	100	0	0.37	0.26	125	—	5.5	115	—	surv.	25.8
A33[f]	Trauma	13.0	0	0	0.63	0.40	0.90	110	13.1	0.60	0.03	90	0	0.66	0.0	100	0.5	4.0	80	90	6.4	18.3
"	Test	5.3	0	0	0	0.35	0.10	70	0	0.50	0.13	90	0	0.54	0.22	120	—	4.5	80	—	50.8	18.3
Av.	Trauma						0.44	125			0.07	80			0.05	77			76	71		
Av.	Test						0.13	109			0.10	103			0.17	121			95	—		
Group II Traumatized dogs only																						
KH25[g]	Trauma	17.7	0	0	0	0	0.80	140	3.0	0	0.04	70	0	0	0.0	75	1.0	2.5	70	65	2.5	3.9
A6[f, h]	Trauma	11.0	+	0	0.57	0	0.24	100	5.36	0	0.04	45	1.19	0	0.0	60	0.3	8.0	50	45	8.8	
A7[f, h]	Trauma	11.9	0	0	0	0	0.14	120	5.93	0	0.0	60	1.11	0	0.0	65	0.0	2.5	90	65	3.1	
A14[i]	Trauma	11.2			0	0	0.11	130	0	0	0.006	80	0	0	0.002	63	2.0	2.3	60	50	2.3	0
Av.	Group II						0.32	122			0.002	64			0	66			68	56		

Group III Control crosstransfusion experiments

A11	Control[j]	9.4	+	0	0	1.39	0.79	90	0	0	0.62	79	0	0	0.70	80	3.8	5.0	55	40	6.0	—
A30	Control[k]	8.6	+	+	0	0.42	0.008	60	0	0.37	0.08	90	0	0.51	0.26	105	—	4.8	60	60	surv.	27.7
A32	Control1	7.9	+	+	0	0.37	0.009	50	0	0.43	0.0	30	0	1.23	0.0	55	0.0	4.0	50	35	12.0	10.0
"	Control2	8.6	+	+	0	0.66	0.18	80	0	0.46	0.01	60	0	1.51	0.22	90	0.5	5.0	65	60	surv.	10.0
A34	Control1	6.8	0	0	0	0.34	0.24	85	0	0.64	0.43	95	0	0.35	0.35	110	—	4.0	75	—	15.0	13.8
"	Control2	6.4	+	0	0	0.40	0.22	110	0	0.38	0.17	110	0	0.45	0.07	120	—	4.0	95	—	surv.	13.8
Av.	Group III						0.241	79			0.22	77			0.33	93			67	49		

Group IV Autotransfused dogs

A12	AT	16.4	0	0	0	0	1.28	130	0	0	0.65	95	0	0	0.21	105	5.5	7.5	65	55	7.5	28.5
A14	AT	11.9	0	0	0	0	0.65	125	0	0	0.49	130	0	0	0.09	110	—	8.5	80	—	surv.	60.9
A15	AT	8.5	0	0	0	0	0.75	120	0	0	0.32	100	0	0.56	0.09	90	1.0	5.5	85	75	8.3	41.0
A17	AT	7.4	0	0	0	0	0.10	95	0	0	0.17	90	0	0	0.02	70	2.0	3.5	70	65	3.5	19.3
A23	AT	6.8	0	0	0	0	0.08	125	0	0	0.20	100	0	0	0.19	115	—	5.0	90	—	10.0	29.7
A24	AT	8.0	+	+	0	0	0.08	100	0	0	0.14	100	0	0	0.06	120	—	5.5	95	—	surv.	33.0
A26	AT	8.5	0	0	0	0	0.07	140	0	0	0.09	115	0	0	0.03	90	3.5	5.0	85	80	surv.	28.9
Av.	Group IV						0.21	119			0.29	104			0.10	100			81	69		

[a] 0 = no reaction, + = drop in mean arterial pressure.

[b] 0 = no cessation; + = cessation of urine flow.

[c] Control period is from start of crosstransfusion until zero time, i.e., until release of compression tubes from trauma dog. In case a crosstransfusion reaction occurred, it is from the second starting of the crosstransfusion, after return of the mean arterial pressure to normal, to the release of the compression tubes. In the case of control crosstransfusion experiments and autotransfusion experiments zero time is taken as 45 minutes after start of the crosstransfusion. or 45 minutes after the second starting in case a crosstransfusion reaction occurred. MAP = mean arterial pressure.

[d] Measured from zero time. Surv. = survived indefinitely.

[e] Where urine flow did not cease this is the lowest MAP recorded in the experiment.

[f] Blood obtained from vena cava of trauma dog.

[g] Traumatized and crosstransfused with a dog whose ureters were ligated.

[h] Traumatized and crosstransfused with a dog whose urine was reinfused. (These experiments will be described further in another publication.)

[i] Traumatized only—not crosstransfused but given heparin.

[j] Crosstransfused with a dog whose urine was continuously reinfused.

[k] Urine collected and measured on only one of the two dogs.

arterial pressure had fallen on the average to 65 mm. of mercury when urine flow ceased.

The effect of trauma is illustrated in figures 1 and 2. In figure 1 (KH32) the trauma dog had, immediately after release of the tubes, a transitory fall in mean arterial pressure to 55 mm. Hg with a diminished urine flow. The pressure quickly returned to a level of 75–85 mm. Hg, which was maintained for 30 minutes. During this time urine flow continued but at a diminished rate. The

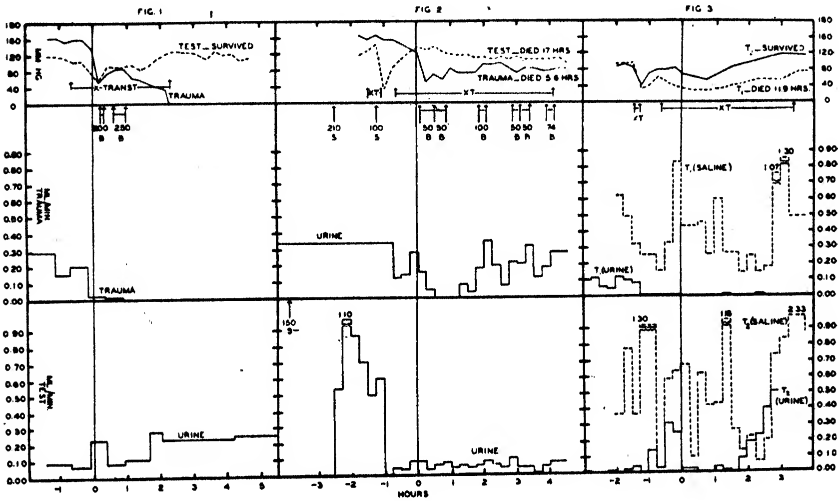

FIG. 1. URINE FLOW AND MEAN ARTERIAL PRESSURE in a normal test dog crosstransfused with a dog subjected to ischemic compression trauma. *TEST* = test dog; *TRAUMA* = traumatized dog; *X* – *TRANS.* = period of crosstransfusion; 200 and 250*B* = ml. of blood transfused into trauma dog; block graph = rate of urine flow, upper of trauma dog, lower of test dog. Abscissal scale = time in hours; ordinate scales = arterial pressure in mm. Hg, rate of urine flow in ml/min.

FIG. 2. SIMILAR TO FIGURE 1. PERSISTANCE OF URINE FLOW in traumatized dog. X–T = periods of crosstransfusion; upper S = saline and B = blood transfusions in ml. given to trauma dog; lower S = saline given to test dog.

FIG. 3. URINE FLOW IN TWO NORMAL DOGS crosstransfused with each other. T_1, T_2 (saline) = rate of infusion of saline into test dogs; T_1, T_2 (urine) = rate of urine flow in test dogs.

pressure declined to 65 mm. Hg during the next 15 minutes and continued to fall until death of the animal. Urine flow ceased with the beginning of the second drop in pressure. The apparent decline in urine flow preceding the release of the compression tubes is due to overlap of the collection period, which was 30 minutes in this experiment.

Figure 2 (A28) illustrates the resumption of urine flow in the trauma dog when the mean arterial pressure was elevated by the infusion of blood. Immediately after release of the tubes the pressure dropped to 55 mm. Hg and then rose during

the next 45 minutes to 80 mm. Hg. Fifteen minutes after the initial drop, the urine flow stopped and no urine was collected for 45 minutes, i.e., until 15 minutes after the pressure had returned to 80 mm. Hg. The arterial pressure then remained above 80 mm. Hg and urine flow continued at a good rate throughout the remainder of the period of observation.

Urine flow in test dogs. Five normal dogs were crosstransfused with traumatized dogs (table 1, group I). Three received saline infusions throughout the period of crosstransfusion in order to provide urine flow adequate for accurate measurement. In none of the 5 experiments was a decline in urine flow observed which could be attributed to the receipt of any humoral substance from the trauma dog. The results in two of the experiments are reproduced in figures 1 and 2.

In experiment KH32, figure 1, no saline was given. The urine flow of the test dog remained unaffected and in fact improved slightly as the arterial pressure rose during the period of crosstransfusion.

In experiment A28, figure 2, a very large initial urine flow resulted from the initial saline infusion. The flow dropped to zero during the decline in arterial pressure to 35 mm. Hg, which was due to a reaction at the start of the crosstransfusion. Upon cessation of the crosstransfusion the arterial pressure returned to the control level of 120 and the urine flow to approximately 0.08 ml/min., at which levels they remained despite resumption of the crosstransfusion and the release of the compression of the hind legs of the trauma dog.

Effect of crosstransfusion reaction. In 10 of the 26 dogs subjected to crosstransfusion or autotransfusion (table 1, groups I–IV), the mean arterial pressure dropped abruptly during the first 15 minutes of crosstransfusion before release of the compression. In each of these the crosstransfusion was stopped and within 30 to 45 minutes the arterial pressure had returned to, or almost to, the previous level. Upon again starting the crosstransfusion, there was no further drop in the mean arterial pressure. The urine flow usually diminished abruptly during the decline of arterial pressure but returned to control levels with the restoration of the arterial pressure. Because of the abruptness of the decline, as shown in figures 2 and 3, it is not possible to state precisely the arterial pressure at which urine flow ceased. The lowest pressures reached in those experiments in which urine flow did cease were: A28—40; A30—40; A32—40; A24—50 mm. Hg. In the remainder of the experiments in which a crosstransfusion reaction occurred, urine flow did not cease during the period of hypotension. The lowest arterial pressures reached in these experiments were: A29—55; A31—100; A34—30; A6—30; A11—75 mm. Hg.

Urine flow in control experiments. The effect of the crosstransfusion procedure alone upon the animal's urine flow was studied in 13 dogs in the two types of control experiments, as indicated in the Methods section. In 6 of these no change in mean arterial pressure or urine flow was observed. In 7 of these animals a considerable drop in the mean arterial pressure occurred, and at these times urine flow ceased. The data from these experiments are included for comparison with the trauma dogs. Table 1 demonstrates that the level of the

arterial pressure at which urine flow ceased in the control animals was not significantly different from that at which urine flow ceased in the various trauma dogs.

In figure 3 the effect of a decline in mean arterial pressure in a pair of control test animals (experiment A32) is illustrated. The mean arterial pressure in T_2 declined steadily from 80 mm. Hg 15 minutes before zero time to 55 mm. Hg one hour later. During this time urine flow diminished and finally ceased for 30 minutes. Urine flow resumed when the arterial pressure returned to 65 mm. Hg and continued to increase as the pressure rose. The arterial pressure of T_1 declined to 35 mm. Hg during the crosstransfusion reaction; for the ensuing $4\frac{1}{2}$ hours the pressure never rose above 60 mm. Hg. Practically no urine flow occurred from the onset of the crosstransfusion reaction to the end of the experiment.

DISCUSSION

Humoral vasoconstrictors. Corcoran, Taylor and Page (3) observed that in shock, urine flow was reduced approximately proportionately to renal blood flow and that the latter was decreased proportionately more than the arterial pressure in the denervated kidney. They concluded that the observed phenomena were due to the presence of a humoral vasoconstrictor. Goormaghtigh (8) found in 4 cases of renal crush syndrome in man glomerular ischemia, enlargement of juxta-glomerular apparatus and hypertrophy and hyperplasia of preglomerular arterioles. These findings were similar to changes he found in the Goldblatt type of experimental hypertension. He attributed the arteriolar spasm at the vascular pole of the glomerular tuft to liberation of a vasopressor substance.

In none of the 5 test dogs which were crosstransfused with traumatized dogs (table 1, group I) was a drop in urine flow observed which could not be attributed to a decrease in mean arterial pressure. It might be argued that in our experiments the exchange of blood was insufficient. However, the volume of blood crosstransfused in each direction amounted to 2–26 liters (0.2 to 3.4 times the body weight of the test dog). Furthermore, in 3 experiments the blood from the traumatized tissue, returning by way of the vena cava, passed through the test dog before returning to the traumatized dog. It seems unlikely, therefore, that if renal vasoconstriction does occur in the kidney of the traumatized dog, it could be due to a humoral agent.

Reflex vasoconstriction. It is possible that reflex vasoconstriction might contribute to the impairment of urine formation (12). The source of the afferent impulses initiating such reflex might be either the homeostatic regulatory endings in the carotid sinus and aortic arch or the traumatized tissue itself. Olson, Walker and Necheles (13) did not obtain anuria in their crush experiments in which the mean arterial pressure remained above 90. Anuria did occur in their hemorrhage experiments and in these the mean arterial pressure had fallen to approximately 60 mm. of mercury when cessation of urine flow occurred. In the latter experiments urine flow was resumed when the mean arterial pressure had been restored to 80 to 90 mm. of mercury. They state, however, that later,

after some $6\frac{1}{2}$ hours, urine formation ceased in the crush experiment, even with mean arterial pressures of 90 and above. Keele and Slome (14) stated that marked reduction in renal blood flow occurred after release of a 4- to 5-hour limb ischemia, and that the reduction was greater than that which occurred when the arterial pressure was lowered to a similar level by hemorrhage.

On the contrary, in our experiments there does not appear to be any significant difference between the level of the mean arterial pressure in the trauma experiments, groups I and II, and that found in the control experiments, groups III and IV, at which urine formation ceased. It is possible then that reflex afferent renal arteriolar vasoconstriction occurs, but, if so, it does not appear to be any more prominent in those experiments involving compression of tissues than in those in which hypotension occurs in the absence of tissue damage.

Interpretation of vasoconstriction. Lauson, Bradley and Cournand (1) and Corcoran and Page (2) observed that in shock the renal blood flow was reduced proportionately more than the arterial pressure. They concluded that vasoconstriction had occurred presumably in the afferent arterioles of the kidney. Selkurt (15) observed that in the presumed absence of change in intrinsic vasomotor tone, renal blood flow is reduced proportionately less than arterial pressure. This observation would tend to confirm the conclusion that, when blood flow is reduced more than pressure, vasoconstriction must have occurred. However, his finding is contrary to that which would be expected on physical grounds from the flow characteristics of a particulate suspension similar to blood in distensible vessels (16) and also is not in agreement with observations on the blood flow in the hind limbs (17, 18). In view of this, we believe that caution should be used in interpreting a given reduction in urine formation or renal blood flow, when accompanying a decline in arterial pressure, as necessarily meaning that renal vasoconstriction has occurred.

Relationship to mean arterial pressure. As mentioned above, Olson *et al.* (13) obtained an initial cessation of urine flow at mean arterial pressures of approximately 60 mm. Hg. As demonstrated in table 1 we also found that under all conditions urine formation rarely ceased at mean arterial pressures above 65 to 75 mm. Hg and rarely persisted when mean arterial pressure declined to 55 to 65 mm. Hg. Thus it appears that 60 to 70 mm. Hg (referred to zero at the level of the carotid artery with the animal level in a supine position) may represent a critical level below which glomerular filtration and urine formation may not be maintained.

Effects of a period of anuria upon recovery of urine formation. Corcoran and Page (19) state that when renal vasoconstriction is present for a considerable period of time, anuria may persist in spite of the restoration of and blood flow blood pressure, possibly due to intrarenal precipitation of myoglobin. Our results as illustrated in figures 1–3 and in table 1 were similar; but more or less identical results were obtained in both traumatized and control animals. Furthermore in one traumatized animal, figure 2, urine flow did return upon restoration of mean arterial pressure despite a period of anuria. It would seem probable that the persistence of anuria after a period of hypotension might be

secondary to renal ischemia per se as proposed by Selkurt (20, 21) rather than to precipitation of hemoglobin or other substance in the renal tubules.

Toxic effects; (*a*) *upon renal tubules.* Eggleton, Richardson, Schild and Winton (4) found that after a crushing injury to the limbs of anesthetized dogs urine flow and creatinine clearance were both markedly diminished. Although urine flow could be restored by diuretics, the creatinine clearance did not increase. Lowering of mean arterial pressure with histamine did not cause the creatinine clearance to remain lowered after restoration of mean arterial pressure, whereas, in the crush syndrome, even with restoration of the mean arterial pressure by infusions, the creatinine clearance remained diminished. They believe that the reduced creatinine clearance may be due to diffusion of the creatinine backward into the blood stream through damaged tubular cells and that the effects are due to toxic agents rather than to tubular blocking. However, if the diminished creatinine clearance were due to the diffusion of creatinine through damaged tubules, the creatinine clearance probably should increase during the period of increased urine flow induced by the diuretics. This was not the case.

(*b*) *By production of tubular blockage.* Bing (5, 6) was able to induce a reduction in urine formation, renal plasma flow and glomerular filtration rate only by the administration of methemoglobin to dogs rendered severely acidotic with intravenously injected ammonium chloride. He obtained no appreciable effect from myoglobin or hemoglobin in either normal or acidotic dogs or from methemoglobin or hemoglobin in normal dogs. The reduction in urine flow with methemoglobin plus acidosis began after several hours and became more severe in the ensuing 2 to 5 days. Microscopic examination of the kidneys showed hydropic degeneration of the proximal convoluted tubule, cellular necrosis in the distal tubule and plugging of the collecting tubules with hyalin and occasionally pigmented casts. Distension of the collecting tubules and glomerular damage were not found.

Bywaters and Dible (7) observed in patients suffering from traumatic anuria various degrees of desquamation, regeneration, necrosis and fibrosis of the tubular cells with the greatest severity in the ascending limb of the loop of Henle and the distal convoluted tubule. Pigmented casts were frequently seen, but hyalin casts were also quite frequent. They believe that circulating myoglobin plays a considerable part in the observed anatomic changes.

In our experiments none of the normal test dogs crosstransfused with traumatized animals showed any evidence of delayed cessation of urine flow such as has been described in the crush syndrome. Evidently, if plugging of the tubules by precipitated myoglobin, methemoglobin or other substances actually plays a part in this phenomena, it must require concomitant reduction in urine formation. From the descriptions given in the literature, it seems possible to us that the renal crush syndrome may represent a change in the distal tubule's permeability with excessive reabsorption of tubular constituents. Our experiments appear to demonstrate that, if such is the case, it is unlikely that it can be produced in the dog by any substance released as a result of a severe 6-hour period of ischemic compression of a considerable mass of muscular tissue.

In earlier experiments (9) it had been noted that urination practically never occurred in dogs dying of ischemic compression shock within the first 12 to 24 hours after release of the compression. Dogs that survived longer than this rarely urinated in the first 12 to 24 hours after release of the compression tubes, and the first urine was dark brown and contained pigment which gave an absorption spectrum characteristic of myoglobin. None of these dogs, however, showed the progressively developing anuria ending in death, which is said to be characteristic of the renal crush syndrome (7).

SUMMARY

Urine formation was measured in dogs subjected to ischemic compression trauma and in test dogs crosstransfused in pairs with the traumatized dogs. No impairment of urine formation was observed in the latter which could be attributed to the operation of a humoral factor received from the traumatized dogs. Since cessation of urine flow occurred at the same mean arterial pressure in control and traumatized dogs, it was concluded that lowering of the mean arterial pressure together with homeostatically induced renal vasoconstriction were probably sufficient to account for the cessation of urine flow in the traumatized dogs.

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TOLERANCE TO HEAT AND DEHYDRATION IN SEVERAL SPECIES OF MAMMALS

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Received for publication October 6, 1947

Tolerance to hot atmospheres differs significantly among species and individuals. What physiological characteristics are related to this diversity? What kinds of resistances to heat are crucial, and how do these resistances fail in heat death? In particular, how do changes of water content of the animal body modify heat tolerance?

Tolerance to heat became susceptible to study following the development, about two centuries ago, of thermometers having permanent scales. At that time various animals were experimentally exposed to warm atmospheres until death ensued (1). It was early noted that men in hot environments had body temperatures lower than the air temperatures around them. How this was possible was first enunciated by Benjamin Franklin (2) on the basis of various observations upon evaporative cooling. The importance of moisture content of the air in determining whether an atmosphere could be tolerated was soon realized, although it has since been found that it is not important in all species. Rectal temperatures of various species of animals exposed to warm air were measured by Delaroche (1, 3); his results indicated that in the rabbit, guinea pig and pigeon, internal temperatures of 42° to 45°C. were lethal.

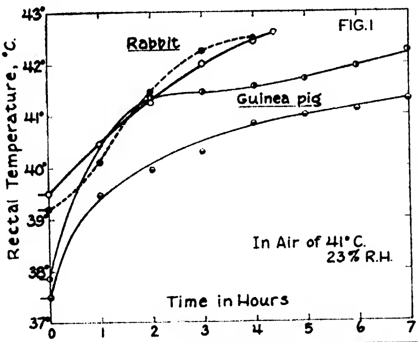
The present report extends to certain laboratory mammals the quantitative information on heat tolerance now available for man (4). The occurrence of fatal heat stroke in man, especially in time of war (5, 6), called for study of the comparable processes and circumstances that are lethal to animals.

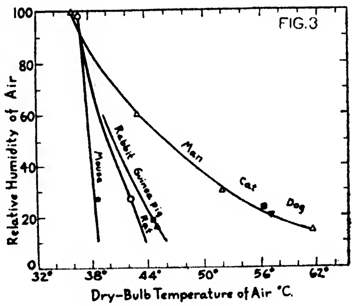
The experiments here reported were done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Rochester. Help was rendered by J. W. Smith and occasionally by other members of the laboratory. J. J. Kelly initiated the experiments on dogs. Preliminary abstracts of the investigation were previously published (7, 8).

Heat toxicity. Laboratory mammals of 6 species—dog, cat, rabbit, guinea-pig, rat, and mouse—were exposed to hot dry atmospheres. Individuals were confined in cages within the hot room. Air was continually taken from outdoors, heated over steam coils and spread through the room. The wet-bulb and dry-bulb temperatures were not uniform throughout the room but were always ascertained at the cages.

In the early hours of exposure the rectal temperatures, measured with clinical mercury thermometers, increased markedly (fig. 1). In atmospheres that were not too hot they often reached or nearly reached a steady state, as here shown for

guinea-pigs; otherwise they continued to rise until the animals were transferred to cool atmospheres, as shown for rabbits. In some species the steady state was achieved by intensification of evaporative cooling through panting. The body was thereby depleted of water, and after several hours the dehydration of the body often became a significant factor in the resistance to heat.





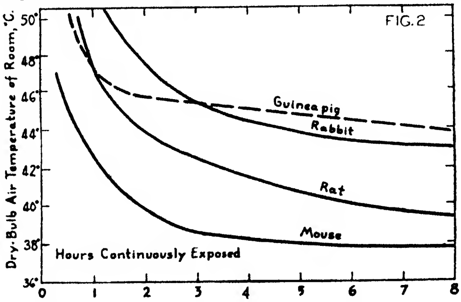


FIG. 1. COURSES OF RECTAL TEMPERATURES in two rabbits and two guinea-pigs simultaneously exposed to warm air. The latter resisted the rise to near-lethal temperatures during the entire day.

FIG. 2. APPROXIMATE MEDIAN TOLERANCES of four species exposed to air of diverse high temperatures.

FIG. 3. APPROXIMATE MEDIAN TOLERANCES, for 3 hours of continuous exposure at rest, to air of diverse temperatures and humidities. The data for man were based upon the onset of exhaustion, not death, and are taken from McConnell and Houghten (24). The other points at high humidity are taken from Macleod (25) for rat, and Mayer and Nichita (26) for rabbit.

When animals were judged to be near the limits of their endurance of heat, they were withdrawn from the hot room to a comfortable room. Many recovered permanently from the state of distress; others died. In this way the tolerance times were ascertained for each of several air temperatures, and for each species an array of points was obtained on the coordinates of figure 2. By drawing a smooth curve between the points for animals that did not survive and for those that did survive, median exposure-time curves were obtained for 4 species, despite the variations among individuals. Moderate amounts of

dehydration prevailed in these tests; in a few individuals water was injected without apparently influencing their heat tolerance. Surviving individuals were used repeatedly; their susceptibilities were not obviously different from those of the uninitiated.

For dog and cat the corresponding curves were not fully ascertained; it was found that 2 cats attained a near-limiting hyperthermia in an atmosphere of 58°C. and 23% relative humidity in only 1.3 and 2.0 hours, and a dog in 2.5 hours, while the same and other individuals tolerated 56°C. for over 3 hours. Dog and cat were also shown by Robinson and Lee (9, 10) to have about the same limits of tolerance for warm air.

The differences of tolerance among the species in the first hours of exposure are related in part to body size. The smaller species and individuals accumulate heat in the body much faster than the larger. But by far the predominant

TABLE 1. *Median tolerances to hot dry atmospheres in five species*

SPECIES	NUMBER OF EXPOSURES	NUMBER LETHAL	MEAN BODY WEIGHT	ASYMPTOTIC AIR TEMPERATURE	$P = (T - A)H$[1]
			kgm.	°C.	
Rabbit	31	5	2.7	41.7	10.5
Guinea-pig	38	11	0.55	43.9	3.4
Rat	32	10	0.27	38.6	10.8
Mouse	19	3	0.023	37.2	5.3
Man[t]	6	0	69.0	59.4	6.0

[1]T = Actual air temperature, °C.
A = Asymptotic air temperature, °C.
H = Median lethal exposure time, hours.
[t] Data from McConnell and Houghten (24), relative humidity 15%.

factor of tolerance is the evaporative cooling of the body. Dog and cat open the mouth, salivate and pant. Guinea-pig and rabbit pant less effectively without opening the mouth. Rat and mouse accelerate the breathing very little. It is not true that all homeotherms either pant or sweat. Though sweat glands are present in the skins of most mammals, no functional sweating over the general skin surfaces is definitely known to occur in response to heat in any of the species here studied. The animals quite commonly wet the fur by licking it or by immersion in any water available.

The curves of figure 2 are all hyperbolas, within the limits of error of their placements. One asymptote of each hyperbola is zero time of exposure; the other is a temperature that may be ascertained empirically (table 1). The height of this asymptotic temperature above 37°C. is an approximate measure of the effectiveness of evaporative cooling in the species.

The importance of evaporative cooling is revealed by comparing the tolerated air temperatures when the air is saturated with moisture and when it is unsaturated (fig. 3). In saturated air evaporative cooling is approximately nil. The mouse is scarcely helped by having dry air instead of moist; the guinea-pig is moderately better off, and the cat is able to withstand 20° higher Centigrade

temperature at 20% humidity than at 100%. Comparison of species shows that, in the dog and cat, cooling by panting helps them to resist warm atmospheres about as effectively as does sweating in man. This fact suggests that in the warmer atmospheres an effective temperature scale would be about the same for dog and cat as it is for man.

The bodily changes in animals subjected to near-lethal atmospheres were followed in terms of rectal temperatures, pulse rates and breathing rates. The best single indicator of the approach to death was found in the rectal temperatures. But each species differed in the maximal rectal temperatures that could

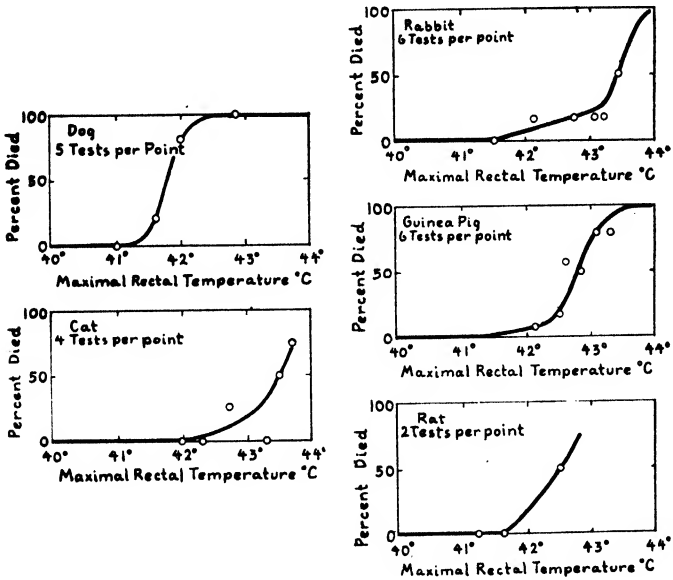


FIG. 4. MORTALITY IN RELATION TO MAXIMAL RECTAL TEMPERATURES among animals exposed to warm atmospheres and dehydration. Individuals were grouped in equal numbers along the abscissae.

be survived. This fact was ascertained by recording the highest temperature found during each exposure and then finding the fraction of the individuals attaining that temperature which subsequently died. The results (fig. 4) can be compared in terms of the temperatures that were fatal to half of the exposed individuals. For a few minutes rabbits could endure 43.4°, rats 42.5° and dogs only 41.7°C. These significant differences evidently characterize certain tissues that are crucial to survival; possibly these tissues are central nervous or circulatory.

The variations in lethal rectal temperatures within any one species are not related to any known factor. Previous exposures appeared to be of no consequence. Dehydration of the body did not induce death at any lower rectal temperature.

Heart frequencies increased moderately as the rectal temperatures rose. The data are most complete for the dog but extend to all species. However, no early sign of approaching death was found in the heart frequencies. Breathing frequencies promptly augmented when animals came into the hot atmospheres and were maintained throughout exposure. For the larger species, complete data upon breathing frequencies have been reported by Lee (11, 12) and Robinson and Lee (9, 10, 13).

The mode of heat death was similar among the species. If kept in the hot room the animals became prostrated. The moment when the heart stopped beating could not be predicted, however. If removed from the hot atmosphere the animals often apparently recovered posture and responses. But death might ensue some hours later, as was found in dogs by Hall and Wakefield (14). Death may be due to a tissue damage (15) that spreads chemically through the circulatory system (16); but it is possible that not all heat deaths are similar. Continued gasping was the most usual sign of heat injury. Occasionally repeated convulsions developed, especially in rabbits; sometimes a single terminal convulsion prevailed. Equally often death was in coma. Wegman (17) concluded

TABLE 2. *Delay in death following heat shock*

SPECIES	DELAYS IN HOURS*
Dog	26, 19, 17, 2, 2, 1
Cat	26, 22, 10, 2, 2
Rabbit	6, 4, 3, 1
Guinea-pig	2, 2, 2, 1, 1, 1

* Hours since animal was removed from hot atmosphere.

that convulsions were rare in cats when the rise of body temperature took many hours.

Delayed death was observed in 4 species (table 2). Evidently its occurrence depended upon the degree of injury that had been produced during exposure to heat. Often, in the first hour or two, dogs were able to drink and thus recover in part from the dehydration. No measure that was instituted, such as cooling or administration of fluids, appeared to influence the progress toward death, though more careful search may uncover such a measure. Usually prostration increased and paralysis spread. Obviously any measure that could shorten the period during which the tissues were subjected to critical temperatures, namely rapid cooling, may be preventive, as it is believed to be in human heat stroke. Much remains to be learned about the injuries that occur in heat stroke.

In animals that survived exposure to heat, subsequent low temperatures were occasionally found. In cats, rectal temperatures of 33.5° and 35.0°C. resulted. In guinea-pigs an hour or two after exposure ceased, rectal temperatures of 36.7° and 36.9°C. occurred. These results suggest that temperature regulation was temporarily deranged by extreme exposures to critical high temperatures.

In general, the rectal temperature was the only index to the onset of heat

stroke that was found useful in laboratory mammals. For each species the danger zone of rectal temperatures was different. Tolerance limits to heat were therefore set *a*) by the capacities for resisting the rise of body temperature and *b*) by the deep temperature that was consistent with life.

Dehydration. The chief object of the experiments was to find the effects of desert dehydration upon heat tolerance. Whereas in man or dog a few hours of exposure to dry heat produced a considerable loss of body water through evaporation, in rat or rabbit the loss of water was small indeed. The latter species avoid the dangers of dehydration but miss the blessings of evaporative cooling.

Evaporation of water continued at a constant rate for hour after hour in a constant atmosphere. In the warmest atmospheres that could be endured for 8 hours, the losses for all species were 1 to 2% of the body weight per hour. The rates did not diminish as the body became dehydrated, as was also found true in man (18). Correspondingly, the breathing rates were not significantly diminished during panting with dehydration.

If an animal that is evaporating water at the rate of 1% of its body weight per hour and has a steady body temperature should suddenly stop evaporating, its mean body temperature would rise 7 Centigrade degrees per hour, computation shows. Correspondingly, it could cool at this rate after transfer to a comfortable environment if the evaporation rate did not decrease. Actually among 14 such transfers of cats, early rates of rectal cooling of 9, 7 and 6 Centigrade degrees per hour were observed, and 8 more of 4 to 5.5 degrees per hour occurred. The initial stages of recovery from hyperthermia are made rapid by the same means that hyperthermia itself is resisted, namely, evaporative cooling. The enormous quantity of heat regularly dissipated by this means to the hot atmosphere has largely come into the body from the environment, for the internal production of heat does not reach the equivalent 5.8 Calories per kilogram per hour in the species here studied.

The course of dehydration could be followed in the dog in terms of serum concentration (fig. 5). As in man, the refractive index increased, often with intervention of an initial decrease of concentration. By the time 10% of the body weight was lost through the evaporation of water, about 25% increase in serum concentration occurred, also as in man. This increase of concentration may represent a corresponding diminution in plasma volume. Far from receiving support from the extravascular volumes, the plasma loses water out of proportion to its fraction of the whole body.

Ultimately the circulating plasma volume apparently became inadequate, for the pulse rate increased and the body cooling was limited by the failure of the heat to reach the body surface from the interior. As long as drinking water was available to the dog, none of these extreme changes occurred.

The dehydrated dog became intolerant of heat. Rather suddenly the rectal temperature increased (fig. 6). This failure to cool was not due to decrease in rate of evaporation, for the panting did not diminish and the tongue was still wet. Moreover, neither the promotion of more salivation by pilocarpine nor the antifebrile action of acetanilide prevented the explosive rise of body temperature

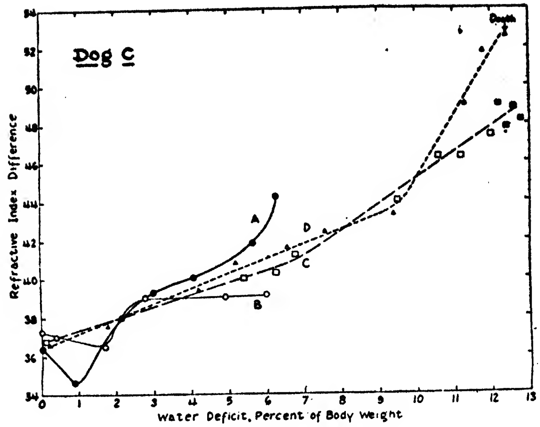


FIG. 5. SERUM CONCENTRATION, in terms of the difference of refractive index between serum and distilled water in arbitrary units. *Dog C* during four exposures to air at 51°C. without water to drink. In exposure C the four black points were obtained after the animal had cooled in a comfortable room. In exposure D the rectal temperature rose explosively after 11% of the body weight had been lost, and the animal did not survive.

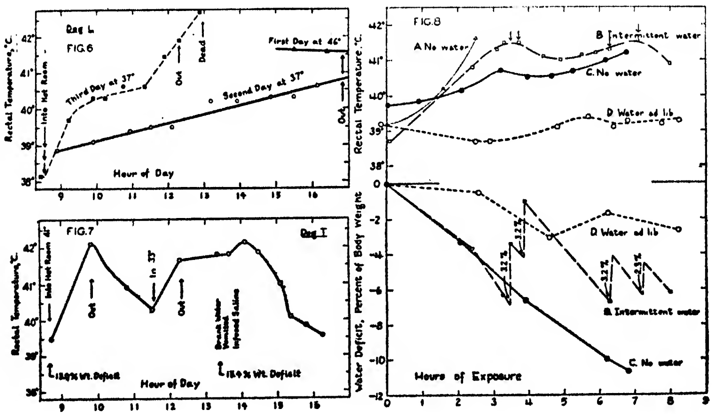


FIG. 6. COURSE OF RECTAL TEMPERATURE in *dog L* exposed, on three successive days, to warm atmospheres as indicated. Water was not allowed for the 52-hour period of the experiment. Explosive rise of rectal temperature developed on the third day.

FIG. 7. COURSE OF RECTAL TEMPERATURE in *dog T* exposed, on the second consecutive day without water to drink, to warm atmospheres as indicated. The animal died 17 hours after it last came out of the hot room.

FIG. 8. COURSES OF RECTAL TEMPERATURE and of water deficit in *dog J* exposed to warm atmospheres on four separate days. Without water to drink (A, 58° air; and C, 56° air) hyperthermia developed at diverse rates; with water ad libitum (D, 56° air) hyperthermia did not develop; intermittent allowances of water (B, 48° air) reduced it after it had developed.

(table 3). Apparently, the circulatory inadequacy left the deep tissues warm and the mouth cool. It is probable that this inadequacy was due to the poor venous return of blood which resulted from loss in volume of blood during dehydration.

The sensitivity to heat that was brought on by dehydration soon reached a point such that the deep temperature was not reduced even when the dog was transferred to an atmosphere cooler than its body (fig. 7). Only in air of less than 20°C. were dogs assured of adequate cooling. During dehydration in the cold, no dog was followed in a comparable manner up to the point of circulatory failure, for water loss then becomes very slow indeed, and refusal of dry food turns the animal's state into one of inanition. Keith (19) and Wierzuchowski (20) rapidly secured equivalent amounts of dehydration without hyperthermia in dogs by the intravenous infusion of sucrose or glucose. Those dogs also were not carried into circulatory failure.

TABLE 3. *Explosive rises of rectal temperature*

DOG NO.	AIR TEMPERATURE	WATER DEFICIT	RATE OF DEHYDRATION	RATE OF RISE RECTAL TEMP.	MAXIMAL RECTAL TEMP.	SURVIVAL TIME IN COOL ROOM
	°C.	*% of weight*	*% of weight/hour*	*°C/hr.*	°C.	*hours*
T	41°	13–14	0.6	2.3	42.1°	17
	33°	14–15	0.3	1.7		
Y	48°	12–13	1.4	1.6[1]	41.7°	2
L	36°	13–14	0.4	1.3	41.9°	0.6
S	54°	16–18	0.9	1.9	41.9°	19
N	47°	15–16	2.4	1.1[2]	41.9°	26

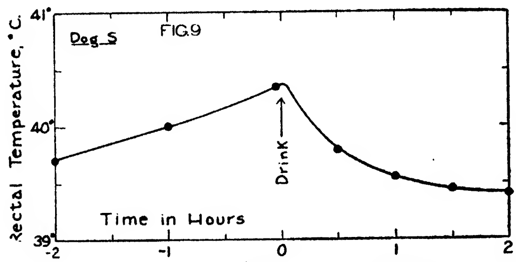
[1] Given pilocarpine (1 mgm/kgm) by intraperitoneal injection during rapid rise of rectal temperature.

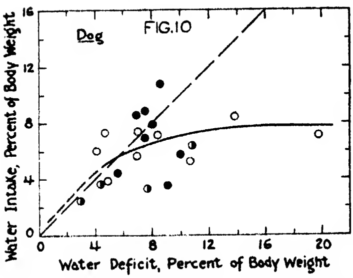
[2] Given acetanilide (50 mgm/kgm) by intraperitoneal injection before rapid rise of rectal temperature.

The explosive rise of rectal temperature developed so suddenly that only a continuous record of rectal temperature allowed the observer to withdraw the animal from the hot atmosphere in time to save its life. Otherwise heat damage was done before the observer became aware of it, and subsequent administrations of water or saline did not aid to repair the damage (fig. 7). A similar amount of dehydration was compatible with life if the hyperthermia was prevented, for 3 dogs recovered from losses of 15, 17 and 20% of the body weight, the last part of which was slowly acquired. Heat intolerance developed regularly when 11 to 14% of the body weight was lost by panting (8 individuals).

Cats withstood slightly more dehydration (17 to 23% of their body weights) before they became sensitive to heat. Even though the cats endured higher rectal temperatures than dogs, their rectal temperatures rose as fast as 1.6 Centigrade degrees per hour toward lethal levels. Their panting also was undiminished.

The voluntary intake of available water by dogs or cats prevented the rise of rectal temperature to the lethal degree (fig. 8). If the water was given early enough, little hyperthermia developed. If it was given after a hyperthermia of 40° to 41°C., the rectal temperature would diminish by as much as a Centigrade degree (fig. 9). Intraperitoneal injection of water into rabbits and guinea-pigs rarely had a significant effect upon endurance in the heat. Only occasionally did their rectal temperatures diminish following an injection. Possibly water has little effect in species of small body size where evaporation and circulation





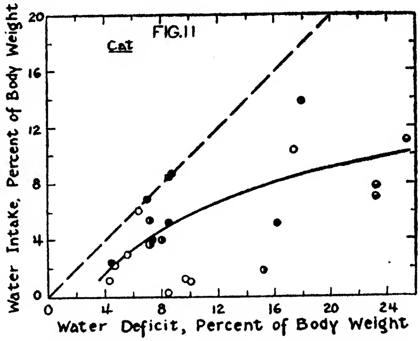


FIG. 9. AVERAGE COURSE OF RECTAL TEMPERATURE in five tests on *dog S*. After losing 7.5% of the body weight by panting for 6 to 7 hours in air of 54°C. and 20% relative humidity, the dog was allowed to drink water of 40°C. temperature for 10 minutes.

FIG. 10. AD LIBITUM INTAKE OF WATER in 21 tests on 3 dogs that had been dehydrated to various extents. Continuation of the line to the left (dashes) represents data previously obtained without exposure to heat (27).

FIG. 11. AD LIBITUM INTAKE OF WATER in 23 tests on 5 cats that had been dehydrated to various extents.

are less important in keeping the body cool. The rôle of water in the preservation of adequate cooling was illustrated in certain species larger than the cat by Flinn (21), Lee and Robinson and Lee (10, 12, and 13).

The species other than dog and cat could be dehydrated to comparable extents only by privation of water (and self-imposed inanition), because of their slow evaporative losses in heat polypnea. Dehydration heat-sensitivity has not yet been identified in them.

In general, the lethal rectal temperature was the same in dehydrated animals as in hydrated ones of the same species. In the presence of dehydration a much

lower air temperature produced the same lethal conditions within the body. Once the critical rectal temperature had prevailed for a few minutes, no known measures could enable the animal to survive indefinitely. Instead it lingered for some hours in a progressively paralyzed state.

Drinking. When dogs were allowed to drink ad libitum while exposed to 48° to 57°C. air temperature, some dehydration developed; only after some hours was water taken as rapidly as it was being evaporated. In two prolonged experiments (32 hours in 48°C.) the dehydration amounted to 6.6 and 7.9% of the body weights. Five cats also drank copiously, allowing only 2 to 6% dehydration to develop in 8 hours. Rabbits, guinea-pigs and rats drank very little in the hot room. Indeed, in them a specific inhibition of drinking may be credited to the hot environment, for animals kept in moderate temperatures without food drank somewhat more water than those in the heat. It must also be recalled that the degree of dehydration produced during 8 hours in the heat (below 8%) in these species, is small compared to those in dog and cat. The dehydration that developed is that which in man we have termed voluntary dehydration (18).

Extensive tests were done to measure how much water animals drink immediately following a stated period of dehydration in the heat without available water. In moderate water deficits dogs tended to drink as much water as they had already lost during heat exposure (fig. 10). They drank equal amounts whether still in the heat or recently removed to a comfortable room. In extreme water deficits less was drunk, the remainder of the deficits being made up later. In cats the results were similar in the large deficits, but in moderate dehydrations smaller drinks were taken than were the deficits themselves (fig. 11). Both species drank proportionately more than men whenever the deficits exceeded 5% of the body weight (18, p. 268). The remaining deficit may be named a terminal voluntary dehydration. How it comes to be larger in man than in other species is at present unknown.

Rabbit, guinea-pig and rat not only developed very small deficits of body water, but in these small deficits they ingested water equal to smaller fractions of the dehydration than dogs did. Whether in large deficits they would drink like cats or like men could not be ascertained.

COMMENT

Comparisons among several species of mammals serve to emphasize the following points concerning tolerance to heat and to dehydration. The species vary greatly in their capacities for evaporative cooling and in the tissue temperatures that they can endure. These factors together with body size appear to be principal determiners of tolerance to heat.

Dehydration has not been found to kill animals within the limits that were tested, but after it prostrates them it makes them highly sensitive to heat. Observations of Czerny (22) upon immature cats early brought this phenomenon to light. Prevention of this sensitivity appears to be the important function served by water in animals subjected to desert atmospheres. Dontas (23) found that dogs and rabbits had to be warmed more than usual in order to induce polypnea when they were somewhat dehydrated.

It was once supposed that any animal can be rapidly dehydrated by exposing it to heat. But panting or sweating is not the rule, especially among rodents. Those species that do not pant lose water only about twice as fast in endurable heat as in comfortable temperatures. They cannot be put into water deficits by evaporative losses before they have also suffered significant inanition. Of the mammals studied here or elsewhere, only sheep (12), dog and cat can tolerate at rest as high air temperatures as can man.

The tests of dehydration show that most species allowed water ad libitum do not drink water as rapidly as they lose water. They go into voluntary dehydration. Even the dog does not at one draft make up deficits that exceed 9% of the body weight.

Acclimatization to heat has been demonstrated in rabbit and dog repeatedly exposed to a hot atmosphere (10, 11). Uniform air conditions are required at each exposure; these were not provided in the present experiments, and for this reason acclimatization to heat was not demonstrated with certainty in 2 dogs subjected to serial exposures originally designed to disclose it.

None of the observations reveal in animals any acclimatization to dehydration. One dog was subjected to dehydrations exceeding 6% of the body weight eight times. It became just as sensitive to heat when carried to 13% dehydration as were 8 dogs that had not been dehydrated before. Further, the lethal rectal temperatures were the same in repeatedly exposed individuals, in newly dehydrated individuals, and in little dehydrated individuals that were unduly heated. No better means is known at present of protecting a mammal against heat than administration of water.

SUMMARY

1. Tolerance times were ascertained in six species of mammals subjected to warm dry air of various temperatures.

2. A consistent sign of lethal conditions was found in the rectal temperature, which had a different median critical value for each species, lying between 41.7° and 43.4°C.

3. Heat stroke appeared to result from hyperthermia that injured unidentified regulatory tissues. Death might be delayed for periods up to 26 hours after animals had been subjected to critical increases of rectal temperature and then cooled.

4. Dehydration through panting was limited in mice, rats, rabbits and guinea-pigs; it was copious in cats and dogs. Evaporation proceeded just as rapidly after considerable dehydration as in water balance.

5. Dehydration concentrated the plasma and by inference depleted the plasma volume, and presumably by limiting the circulation, rendered the animal very sensitive to warm atmospheres. An explosive rise of rectal temperature developed through failure of the circulation to transport heat to the surface.

6. Animals that were allowed water ad libitum did not drink enough to maintain their body weights while they were in hot atmospheres. If deprived of water they (except dogs in water deficits of less than 9% of the body weight) subsequently drank less than enough to recover their body weights.

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SWEATING PATTERNS IN THE SKIN FOLLOWING INJECTIONS OF MECHOLYL[1]

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Received for publication October 13, 1947

While studying the direct stimulating action of certain cholinergic drugs upon the sweat glands, interesting patterns of sweating indicating stimulation of sweat glands at considerable distances (1.0 to 37.0 cm.) from the site of injection were observed. The character of spread of the sweating response suggested that other than strictly nervous mechanisms were involved.

A method for recording individual sweat-gland responses previously reported (1) was used. Mecholyl[2] (acetyl-beta-methyl choline chloride) in concentrations from 1–500 to 1–50,000 was introduced into the skin of the forearm by intradermal and subcutaneous injections with continuous recording of sweating.

During the injection a wheal about 5 to 10 mm. in diameter formed and immediately a few sweat spots appeared around its circumference. From this sharply localized response, sweating rapidly spread in the form of restricted channels for some distance. Oftentimes, channelized processes leading away from the wheal were first outlined by a few sparsely scattered sweat spots, and with each successive record more and more sweat spots appeared until nearly all of the sweat glands in the sharply delineated channel patterns were functioning. As more and more such processes formed and extended far from the site of injection, considerable branching and anastomosing occurred as oblique and lateral connections between the processes. The width of the anastomosing processes varied from 1 mm. to 5 mm., and they were observed in some cases to follow precisely the course of large surface veins. In other cases they appeared to follow the course of invisible channels. In one such instance a narrow band of sweat spots was traced along the course of the cephalic vein from a point on the dorsal surface of the forearm upward until the vein penetrated more deeply into the tissue above the antecubital fossa, 37 cm. from the site of injection.

Spread by these radiating bands was much more prominent and sometimes confined exclusively to the central side of the wheal with 'streaming' in a 'venous' direction. They seldom spread more than 5 to 8 cm. distally from the wheal toward the extremities but rather spread centrally toward the body in the same direction as the venous and lymphatic drainage. Spread by direct diffusion around the wheal remained localized and seldom exceeded a few millimeters (being halted when a transverse or ascending channel was encountered).

Lateral spread was noted with relatively short bands of sweat spots extending laterally and anastomosing with larger channels coursing up (and occasionally

[1] Aided by a grant from United States Public Health Service.

[2] Mecholyl furnished through the courtesy of Merck & Co., Inc.

for short distances down) the arm. Large sweating areas often marked the region of junction between two or more channels, suggesting the presence of relatively large sinuses in such regions.

With this remarkable spread around a localized area of sweating the probability of spread by local reflex pathways was diminished by the restricted character of the sweat patterns. It became evident that the drug was being rapidly transported away from the site of injection by a fluid stream, and since the patterns often followed exactly the pattern of branching or anastomosing veins, the possibility of transport by venous blood arose. Since the sweat glands were being stimulated to activity, however, apparently by direct stimulating action of the drug, and since rapid diffusion of the drug into and out of the relatively heavy-walled veins seemed improbable, such a source for these patterns appeared doubtful. Diffusion through the walls of the lymphatic plexus lying in the immediate neighborhood of the secretory portions of the sweat glands in the corium seemed more likely. The most logical course of convection appeared to be, therefore, via lymphatic channels, the larger vessels of which are superimposed over the larger veins in the skin (2).

An experiment was designed, therefore, to occlude the lymphatic channels and determine thereby whether spread of the drug could be prevented. Accordingly rubber bands attached to strong but fine cord were tied around the arm just central to the injection site (12 minutes, fig. 1). This procedure prevented the appearance of the centrally radiating channels of sweat spots, but distal radiations developed to a somewhat more marked extent than usual. Since the edge of the wheal was closely adjacent to the occluding cord, sweating due to diffusion of the drug was prevented. In order to determine whether sweating could be induced by axon reflex (as defined by Bickford, 3, and Wilkins, *et al.*, 4), bipolar electrodes were placed in the sweating area immediately distal to the occluding string (arrow at 14 minutes and 30 seconds in fig. 1). Prominent and widespread sweating appeared in the previously non-sweating areas in all directions, including those central to the occluding cord, within 30 seconds following the start of faradic stimulation. Therefore, the occlusion did not prevent local nervous spread of sweating. This sweating decreased abruptly when the electrodes were removed with only slight traces remaining at 16 minutes, 30 seconds (fig. 1). The occluding string was cut at 16 minutes, 50 seconds. Sweat spots appeared very quickly in a central direction (within 10–20 seconds) and centrally radiating channels of sweat spots formed in the usual fashion. It is significant that sweating elicited by faradic stimulation and that appearing following release of occlusion assume markedly different patterns, thus differentiating again between nervous spread and the convection of the cholinergic drug by a fluid stream. Within 11 minutes after the occlusion was removed, sweat glands were active at a distance of more than 7.5 cm. from the wheal (28′ of fig. 1). Such patterns were not prevented by mixing the drug with procaine or by previously anesthetizing the area with procaine.

In following the course of two widely separated channels of sweat spots, it was often observed that the two channels would converge to form one large

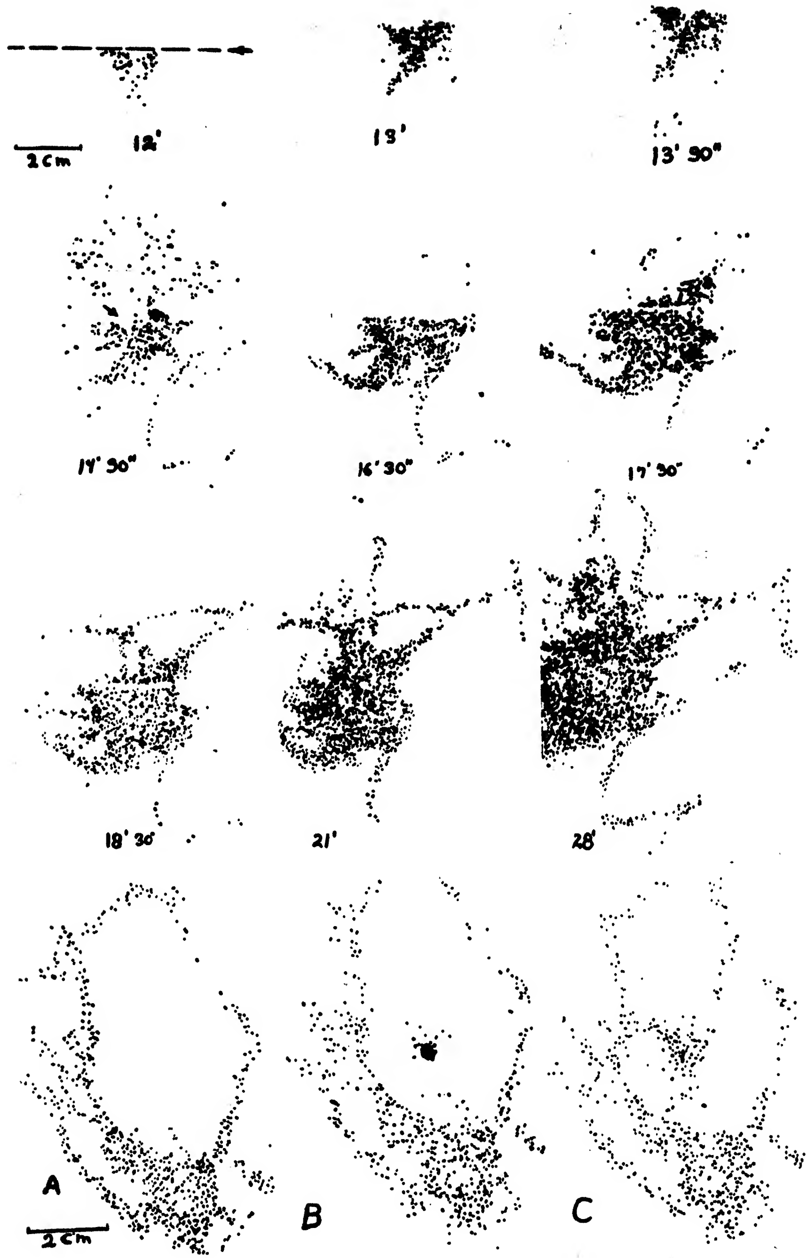

FIG. 1. FOREARM SWEAT RESPONSE to intradermal injection of 0.05 cc. of 0.5% mecholyl immediately distal to a string occluding the cutaneous lymphatic and venous drainage. Numbers on each of first nine records indicate (in minutes and seconds) time course of the experiment. Position of occluding string indicated in first record. The string remained in place until cut immediately following record 16'30". Calibration line indicated on first segment. With string still in place, faradic stimulation applied at arrow in record 14'30".

Segments *A*, *B*, and *C* demonstrate spread of sweating in restricted channels of sweat spots on upper arm. A secondary injection of 0.01 cc. of 1.0% mecholyl in segment *B* induces spread by channels which eventually empty into those originally outlined in segment *A*. Calibration line site indicated in segment *A*.

Photographs of original records retouched with India ink for purposes of reproduction.

channel at some point far from the injection wheal. There would then be relatively large non-sweating areas completely surrounded by narrow bands of sweat spots (segment *A*, fig. 1). Injection of minute amounts of mecholyl into the nonsweating area (arrow, segment *B*) induced a sweat response similar to that in the original, and radiating bands of sweat spots formed and eventually joined the channels already outlined in the periphery (segment *C*, fig. 1). This illustrates the fact that functional sweat glands are present in the large non-sweating area of segment *A*, figure 1, and that they are responsive to direct cholinergic stimulation. The fact that the drug did not flow back into this area via the channels indicated in segment *C* suggest the possibility of valve-like structures located at such bifurcations which prevent significant backflow. Such valves have been identified in the deeper lymph channels by Drinker and Yoffey (2) who state that once lymph enters these channels it can move in only one direction.

It appears that the spread of sweat responses in these experiments is much more rapid than is the flow of lymph in the quiescent limb. As has been pointed out by Drinker and Yoffey, however, massage greatly speeds the flow of lymph through lymphatic channels. Since the method used for recording the sweat responses involves light pressure applied over the entire test area, such rapid flow could be readily explained. These authors also pointed out that even light massage tends to increase the permeability of the lymph vessels. This probably explains the ready diffusion of mecholyl into and out of the vessels and into the immediate environment of the sweat glands, where direct stimulation would be possible throughout the entire length of lymph channels underlying the long bands of sweat spots observed in some experiments. The relatively sharp delineation of sweating from non-sweating areas indicates there is little diffusion of the drug away from the channel areas. On the other hand sweating persists without spread in these areas in the quiescent subject for long periods, suggesting that the stimulating action of the drug continues until it is destroyed by its esterase, or it is dispersed and diluted in the lymphatics until its concentration falls below the threshold for stimulation.

Dilatation of minute blood vessels in the sweating areas was indicated by long narrow erythematous zones. The erythema was sharply confined to the same narrow channels as those outlined by sweating responses. At the termination of an experiment, when the excess iodine was removed from the skin, an erythematous area roughly comparable to the dimensions of the sweating regions indicated dilatation of blood vessels.

Introduction of the drug into large areas of the skin by iontophoresis commonly resulted in similar but less pronounced patterns of dispersion. It has been repeatedly observed in this laboratory, however, that skin blood flow may be increased considerably in areas several centimeters central to the site of iontophoresis of mecholyl. Since the sweating patterns are not as pronounced as observed following cutaneous injections, one might assume that with the strength and duration of current employed in these experiments (0.1 to 1.0% solution at 3.5 to 4 ma. for 10 to 20 minutes with electrodes from 3 cm. by 4 cm. to 4 cm.

by 5 cm.), the drug is too greatly diluted or is not present in sufficient amount to produce extensive sweat gland stimulation at any considerable distance from the wheal.

Since, however, the skin does become somewhat erythematous and the skin pulses increased in size as measured by the photoelectric plethysmograph, it appears probable that at least some superficial spread from site of administration does occur.

The therapeutic implications of these observations in cutaneous vascular disorders appear to be limited by the relatively small spread into areas adjacent to the wheal and the narrow channels of stimulation. Direct diffusion through the intercellular spaces did not appear to be significant. Therefore, although spread through the superficial lymphatic and venous plexuses may result in considerable dispersion of therapeutic agents, the importance of local administration directly into large areas (as by iontophoresis) would seem to be confirmed. It is suggested, however, that in those cases in which electrodes can not be directly applied (as in surface ulcers), considerable benefit may be realized via the mechanisms described here, provided the lymphatic and venous channels remain intact and functional.

SUMMARY

The iodine-starch paper method was used in a study of sweating responses elicited by intradermal and subcutaneous injections of mecholyl (acetyl-beta-methyl choline chloride). Unique patterns of functioning sweat glands following mecholyl injections suggested the participation of cutaneous lymph channels in convection of the drug away from the site of injection. These patterns consisted of narrow channels of sweat spots radiating outward from the wheal, being most prominent in a central direction, and apparently following the course of lymphatic drainage. The probability of nervous intervention in the spread of sweat patterns was negated.

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INFLUENCE OF HIGH FAT DIETS ON ALLOXAN DIABETES[1]

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Received for publication October 6, 1947

Although the dietary control of diabetes mellitus has been less rigorous since the discovery of insulin, regulation of the diet is in many cases still an important consideration. High fat diets were formerly advocated for diabetic patients (1, 2), but today most internists recommend a diet moderately high in carbohydrate and low in fat (3).

Recently, Burn, Lewis and Kelsey (4) pointed out that in alloxan diabetic rats glycosuria disappeared when a high fat diet was substituted for a normal diet containing 10% fat. Furthermore, if a high fat diet was given alternately with a normal diet for a period of time, the diabetes steadily lessened.

The rats used by the aforementioned workers were not severely diabetic and this disorder was not allowed to become well established before the high fat diet was introduced. Inasmuch as it has been found that the blood sugar of diabetic rats having levels similar to those used by Burn *et al.* may decrease spontaneously after a period of time (5), the present study with high fat diets has been carried out with severely diabetic rats in which the diabetes had ample time to become well established.

METHODS

Twenty adult male rats of the Long-Evans strain were used. Eighteen of them had had a severe alloxan diabetes for a period of 1–3 months before the experiment was started. Two rats had only a transitory diabetes.

The composition of the experimental diets is shown in table 1. To provide adequate calories from protein and sufficient vitamins and minerals for the maintenance of normal metabolic processes, the high fat diets were proportionately supplemented with these substances. The animals were allowed to feed ad lib. throughout the experiment, but food intake was carefully determined at the end of each 24 hours. All of the rats were given the basic diet (10% fat) for a control period of three weeks. At the end of this period the fat content of the diet was progressively increased (see table 1) at weekly intervals for as long as the rats could tolerate the diet or until the 60% level of fat was reached. Eight of the rats were sacrificed and certain organs were examined histologically when they had either reached the highest fat levels or the maximum level which they could tolerate. The remaining 12 rats were returned to the basic diet for three weeks.

During the control and experimental periods caloric intake and urine volume measurements and qualitative estimations of urinary acetone bodies were made

[1] Supported by a grant from Hoffman-LaRoche, Inc.

daily. Quantitative blood and urine sugars and urinary acetone bodies were determined on nonfasting rats at weekly intervals; the former by the micro-colorimetric method of Somogyi, the latter by the gravimetric method of Van Slyke.

TABLE 1. *Composition of experimental diets*

		PER CENT OF FAT							
		10	20	30	35	40	45	50	60
Casein	(grams)	180	210	230	240	250	260	270	290
Sucrose	"	320	340	270	235	200	165	130	60
Starch	"	400	250	200	175	150	125	100	50
Crisco	"	50	100	200	250	300	450	500	600
Wesson oil	"	50	100	100	100	100	—	—	—
Salt mixture	"	30	33	37	38	40	42	43	47
Choline	"	2	2.2	2.5	2.6	2.7	2.8	2.9	3.1
Thiamine HCl	(mgm.)	5	5.6	6.1	6.4	6.7	7.0	7.2	7.8
Niacin	"	10	11.1	12.2	12.8	13.3	13.9	14.4	15.5
Pyridoxine	"	5	5.6	6.1	6.4	6.7	7.0	7.2	7.8
Ca pantothenate	"	15	16.7	18.3	19.1	20.0	20.8	21.7	23.4
Riboflavin	"	5	5.6	6.1	6.4	6.7	7.0	7.2	7.8
Haliver oil	(cc.)	2	2.2	2.5	2.6	2.7	2.8	2.9	3.1
Calories/100 grams		450	500	550	575	600	625	650	700

RESULTS

The results shown in table 2 are the individual data for the 12 rats which survived the three periods of study. The three-week initial period, with the basic 10% fat diet, is designated as *I*. All of the values opposite *I* represent averages for the 3 days, a week apart, on which the blood and urinary sugar levels were determined. *HF* represents data for one day at the end of the week when the highest percentage of fat was given. The terminal three-week period, after the high fat diet, is designated by *T*, and these values are averages of the two days, one in each of the final two weeks.

Initial period (*I*). During this period all but two of the rats showed the characteristic symptoms of severe uncontrolled diabetes, except that they excreted only small amounts of acetone bodies. The daily total caloric intake differed from animal to animal but in general was correlated with the severity of the diabetes and the weight of the animal. Discounting daily fluctuations, body weights were either maintained or slightly increased during this period. The urine volumes and urine sugar values could be correlated with the caloric intake; when the caloric intake was high, the urine volume and sugar excretion were high. Blood sugar values did not always follow this pattern because the determinations were not made on animals in postabsorptive condition.

Period when maximum fat was given (HF). In this period the rats, with one exception, decreased their food consumption. These figures are not representative of the entire high fat period because most of the rats were in severe acidosis

TABLE 2. *High fat diet and alloxan diabetes*

RAT NO.		FAT IN DIET	CALORIC INTAKE	WT.	BLOOD SUGAR	URINE VOL.	URINE SUGAR	URINARY ACETONE BODIES[1]
		%	24 hr.	grams	mgm. %	cc/24 hr.	grams/24 hr.	mgm/24 hr.
4	I	10	156	325	605	268	20.6	8.26
	HF	20	141	349	460	231	18.0	272.84
	T	10	147	331	596	281	23.1	9.52
3	I	10	149	343	552	257	25.4	7.09
	HF	20	130	335	498	245	20.0	203.00
	T	10	153	340	627	289	25.2	5.68
2	I	10	132	243	569	238	18.8	6.63
	HF	20	97	242	446	159	11.0	229.40
	T	10	98	243	834	231	18.2	12.00
16	I	10	119	336	684	315	24.8	16.70
	HF	30	116	350	476	197	12.4	370.00
	T	10	95	323	718	230	19.1	23.96
7	I	10	160	314	733	301	25.5	14.78
	HF	30	110	315	426	137	9.5	228.30
	T	10	128	306	701	246	23.7	13.79
15	I	10	137	348	604	315	12.6	26.06
	HF	45	84	375	408	81	4.5	166.00
	F	10	135	383	485	278	10.0	17.08
13	I	10	118	313	596	205	18.8	3.71
	HF	45	70	348	382	37	1.4	99.57
	T	10	93	330	580	108	9.8	6.53
5	I	10	205	338	616	327	27.2	9.06
	HF	50	68	371	292	50	4.0	323.70
	T	10	152	351	562	241	24.3	2.58
6	I	10	192	430	596	313	28.8	12.74
	HF	60	91	465	330	14	3.2	97.70
	T	10	103	440	546	187	18.4	7.60
1	I	10	132	436	510	150	11.9	5.40
	HF	60	63	456	328	34	1.1	1.04
	T	10	49	445	330	41	2.3	0.37
10	I	10	66	346	130	6	negligible	0.22
	HF	60	49	427	90	7	"	0.91
	T	10	55	433	120	3	"	0.72

TABLE 2—*Continued*

RAT NO.		FAT IN DIET	CALORIC INTAKE	WT.	BLOOD SUGAR	URINE VOL.	URINE SUGAR	URINARY ACETONE BODIES[1]
		%	*24 hr.*	*grams*	*mgm. %*	*cc/24 hr.*	*grams/24 hr.*	*mgm/24 hr.*
9	I	10	63	344	126	11	negligible	0.21
	HF	60	67	344	150	7	"	0.30
	T	10	49	356	126	2	"	0.59

I = Initial period when rats received diet containing 10% fat.
HF = Period when rats received maximum fat.
T = Terminal period when rats received diet containing 10% fat.
[1] In addition, the ketonuria in the 8 rats which were sacrificed while receiving maximum fat was as follows:

% fat	*Ketone bodies*
30	202.83
35	256.88
40	178.20
60	115.39
60	42.43
60	3.50
60	22.02
60	8.89

and showed some anorexia. A lower caloric consumption was particularly evident in the animals receiving the higher percentages of fat. The urine volumes and blood and urinary sugar levels were also decreased. This decrease probably resulted from the lower intake of food and of potential glucose.

There was considerable variation in the tolerance to the high fat diets; for instance, on the 20% fat diet certain animals had a severe acetonuria which persisted when the same diet was continued for three weeks. Other severely diabetic animals failed to develop an acidosis with much higher percentages of fat. The ketosis exhibited by the 20 rats on the maximum level of fat is shown in table 2.

It is interesting to note that usually the animals that could not tolerate the higher percentages of fat were more diabetic, as judged by blood and urine studies, than the rats which tolerated the 60% diet. If some of the rats had been given higher percentages of fat, the acidosis would probably have been fatal.

Histological examinations were made of certain organs from the 8 rats which were killed. Increased amounts of osmicated lipoid were found in the adrenals and kidneys over that found during the initial period. The liver also showed increased amounts of lipoid as evenly distributed fine droplets, together with some large clumps of lipoid material. The pancreatic islets showed the characteristic alloxan damage, but some of the islet cells apparently had recovered from the earlier impairment.

Terminal period (*T*). Within a few days after the return to the basic diet there was a rapid decline in the urinary acetone body excretion; in fact, the acetonuria completely disappeared in most animals after seven days. Con-

current with the disappearance of acidosis there was generally some increase in caloric consumption over the high fat consumption level, but in many of the animals that had been on the higher percentage of fat it was not as high as during the initial period. Moreover, blood and urinary sugar levels were markedly increased over levels observed during the high fat regime. At the end of the terminal period 5 severely diabetic animals, which previously had received diets containing 45 to 60% fat, had blood and urinary sugar values below those observed during the initial period; the remaining 5 diabetic animals had glucose levels similar to those found during the initial period.

During the last two weeks of the experiment most of the rats lost much of the weight gained while on the high fat diet, even though they were consuming more calories than during the high fat regime. Furthermore, most of the rats were excreting more glucose in proportion to the caloric intake than was excreted during the initial study period. Both the weight loss and the increased glucose excretion offer indirect evidence that the animals were metabolizing depot fat which had been stored during the high fat period.

When the remaining 12 animals were examined the lipoid distribution in the adrenals, kidneys and liver proved to be similar to that seen in rats during the initial period. The structure of the pancreatic islets was not unlike that seen when the rats were receiving the maximum percentages of fat. In many instances it was impossible to determine the severity of the diabetes by examining the pancreatic islets.

DISCUSSION

The principal question involved here is whether or not a gradual increase of fat in the diet of alloxan diabetic rats will produce a permanent amelioration of the diabetic symptoms as described by Burn *et al.* (4). In the present study, although glycosuria was not abolished, 5 of 10 severely alloxan diabetic rats, which had been given high fat diets for a period of up to 55 days, showed reduced blood and urinary sugar levels (as compared to initial levels) after they were returned to the basic diet for three weeks. Ordinarily when a rat is so severely diabetic, the diabetes usually remains permanently severe (5). Although there may have been some spontaneous recovery of the diabetes, the high fat diet was probably indirectly responsible for the milder diabetic symptoms. During the high fat diet the available carbohydrate and blood sugar levels were reduced, and this apparently gave the pancreatic islands an opportunity to rest (6). In fact, microscopic preparations of the islets from some of the animals which had received the higher percentages of fat seemed to show a greater recovery from the beta cell damage produced by alloxan than the tissue from diabetic rats which had not received the fat diet. Furthermore, a somewhat similar islet recovery has been noted in diabetic rats which had an anorexia of long standing caused by thiamine deficiency (7). Thus it would seem that a prolonged decrease in blood sugar levels in alloxan diabetic rats permits some recovery of the pancreatic islets and, in certain cases, improvement of the diabetic state.

While the rats were receiving the high fat diets their blood sugar levels were reduced. Although not a comparable experiment, Somogyi and Cook (8) found that diets containing high fat rations and restricted amounts of carbohydrate increased the postabsorptive blood sugar levels of diabetic individuals. The fact that the rats were not fasted while the human subjects were in a postabsorptive state may account for the variance in observations.

The acidosis observed in the rats receiving the high fat diets was much more severe than was reported in the studies by Burn *et al.* (4). Two factors may have been responsible for this: *a*) the rats in the present study were more profoundly diabetic and thus more susceptible to ketosis; *b*) the increased amounts of protein per unit of food, which was added to the high fat diets in proportion to the total caloric content, may have aggravated the ketosis. Petrén (2) pointed out that a high level of protein in a diet increases the level of acetone body excretions. However, the dietary protein in the present experiments was maintained at a 16% level of the total caloric content, a percentage within the range recommended by Francis, Smith and Mendel (9) as the amount needed to meet the nutritional requirements of the rat.

The increase in body weight of most of the rats while they were receiving the high-fat diets is probably not the result of growth per se but merely owing to increased fat deposits; the gain was generally lost on the terminal basic diet. Since the rats in the terminal period were excreting more glucose in proportion to the caloric intake than during the initial period, indirect evidence suggests that during the terminal period they were metabolizing the stored fat for energy requirements and excreting more of the potential glucose in the diet.

SUMMARY

High fat diets were given to 20 adult male rats of the Long-Evans strain. Eighteen of the animals had severe uncontrolled alloxan diabetes and two a transitory diabetes.

The amount of dietary fat was increased progressively at weekly intervals for as long as the rats could tolerate the diet or until a 60% level of fat was reached. Tolerance was determined by the amount of ketonuria produced by the diet.

While on the high-fat diet all of the rats had reduced blood and urine sugar levels and most of the animals gained weight. When 10 of these animals were returned to the basic diet for three weeks and the severity of the diabetes was compared with that of the initial basic period, 5 rats showed some improvement in diabetic symptoms. This improvement may have been spontaneous, but more likely it resulted from some recovery of the damaged pancreatic islets which occurred while the animals were on the high fat diets. The weight gain which occurred during the feeding of high-fat was probably caused by an increased deposition of fat.

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VARIATIONS IN THE EFFECT OF ANOXIA ON PERFORMANCE[1]

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Received for publication March 10, 1947

Variations in the effect of anoxia on human performance may significantly alter the relative efficiency of persons working under lowered oxygen tensions. Among the factors known to influence these variations are rate of ascent (1), degree of activity and duration of exposure (2), state of health (3), psychogenic disorders (4) and cardiovascular disease (5). However, when these factors are equalized by the use of suitable subjects and experimental conditions, a considerable range of variation still remains which has been considered to represent individual differences in the underlying physiological adjustments to anoxia (6).

The present study was undertaken to determine under uniform conditions the relationship of anoxic alterations in individual neuromuscular functions to one another and to the mean performance of the subject. Three specific aspects of this relationship were investigated: *a*) the amount by which the same function is altered in different subjects; *b*) the degree to which different functions are altered in the same subject; and *c*) the extent to which single tests may be used to indicate changes in performance.

PROCEDURE

Fifty volunteer male subjects were selected whose age, physical status, diet, and living and working conditions were essentially alike. Each subject was exposed to a series of four ascents in a low-pressure chamber without oxygen to an equivalent altitude of 17,000 feet. From 5 to 12 subjects were accommodated at a time. The 'flights' were spaced approximately one week apart. The rates of ascent and descent were constant. The duration of the flights averaged 2 hours. The subjects were scored on 5 separate tests made in the chamber at ground level and at the end of a period at altitude which varied from 60 to 75 minutes. The subjects were rotated through the tests in the same order each time.

Selection of tests. The selection of tests was guided by two criteria: measurement of a function directly involved in the operation of aircraft and linearity between change in score and variation in effectiveness of the function which the test was designed to measure. In certain of the tests effectiveness undoubtedly was conditioned by relatively independent alterations in more basic factors such as reaction time, accuracy and attentiveness. A quantitative estimate of these factors was not attempted, since a measurement in terms of net accomplishments was considered to possess greater practical significance.

Test battery. Simple arithmetic tests of the type used in AAF cadet classifica-

[1] The contents of this article have been released for publication by the Army Air Forces.

tion centers were employed as a measure of methematical skill. Each test consisted of a series of problems in addition, the score being the number of problems solved correctly in 3 minutes.

Eye-hand coordination was evaluated by the single dimensional pursuit test developed at the AAF School of Aviation Medicine. The test was scored on the portion of a 105-second interval during which the subject kept an irregularly moving white bar aligned with two centering marks. Alignment was accomplished by movement of a control column projecting from the apparatus. The similarity in scores made by the same individuals on successive ascents suggested that the learning factor in the test is small, adapting it to the recurrent study of the same group.

Visual discriminatory ability was estimated by employment of a card used in AAF altitude-training units to demonstrate anoxic effects. The test depended on the ability of the eye to discriminate between text of uniform size and blackness and a background varying from white at the top of the card to black at the bottom. Each line was numbered in linear relation to the blackness of the background. As a consequence, a doubling of the line number corresponded to a halving of the contrast between text and background. The number of the lowest line read was taken as the score.

An adaptation of the Minnesota block test was utilized to indicate manual dexterity. The test was scored on the number of cubical blocks replaced in a square box, one at a time, in 90 seconds. Inspection of successive scores made by the same individuals indicated the existence of a considerable learning factor, which requires that comparisons based on this test be made between groups located at similar points on a learning curve.

The area of the peripheral visual fields was measured by the use of the Lloyd stereocampimeter.

METHODS OF ANALYSIS

The average of the ground level scores made by an individual in a particular test was taken to represent his normal performance. The percentage by which the mean score at altitude varied from this normal score was used to indicate the effect of lowered oxygen tension on the function which the test was designed to measure. Each individual was ranked in the test on the basis of this percentage difference. The averages of these ranks and of the percentage ground-altitude differences in the 5 tests were employed as indices of the degree to which the individual's over-all performance had been impaired by anoxia.

Impairment of group performance at altitude was determined for each test by averaging the alterations in individual performance. The degree of variation within the group was estimated by calculation of the standard deviation and coefficient of variation of the ground-altitude scores differences.

The significance of differences in the degree to which group performance was impaired in the various tests was evaluated by determining the ratio of the observed difference in means to the standard error of the difference between means.

The degree of correlation between individual tests and between each test and

the over-all battery average was determined by application of the Spearman rank-difference formula.

RESULTS

The mean score at altitude made by individual subjects on individual tests varied from +15.6% to −64.4% of the ground level value. Over-all impairment

TABLE 1. *The effects of anoxia on five tests of human performance in terms of ground-altitude scores differences*

TEST	MEAN DECREMENT % AND STANDARD DEVIATION	RANGE %	COEFFICIENT OF VARIATION (V)	STANDARD ERROR OF THE MEAN (S.E.M.)
Cube placing	−7.8 ±8.1	−22.6 to +15.6	1.02	±1.14
Vison demonstration card	−10.9 ±8.4	−25.0 to +5.0	0.78	±1.19
Single dimensional pursuit	−12.1 ±6.9	−29.3 to +1.0	0.57	±0.97
Arithmetic	−21.6 ±11.3	−48.3 to 0	0.52	±1.61
Visual field perimetry	−26.1 ±17.0	−64.4 to ±1.0	0.65	±2.39

Number of subjects: 50.
Number of trials: 4.
Chamber altitude: 17,000 feet.
Time at altitude: 60–75 minutes.

TABLE 2. *The significance of differences in the degree of group impairment in performance on different tests at a simulated altitude of 17,000 feet*

TEST	CUBE PLACING	VISION DEMONSTRATION CARD	SINGLE DIMENSIONAL PURSUIT	ARITHMETIC	VISUAL FIELD PERIMETRY	
Cube placing		3.1	4.3	13.8	18.3	
Vision demonstration card	1.9		1.2	10.7	15.2	
Single dimensional pursuit	2.8	0.8		9.5	14.0	d_M
Arithmetic	7.0	5.4	5.1		4.5	
Visual field perimetry	6.9	5.7	5.4	1.5		

$d_M/S.E.d_M$

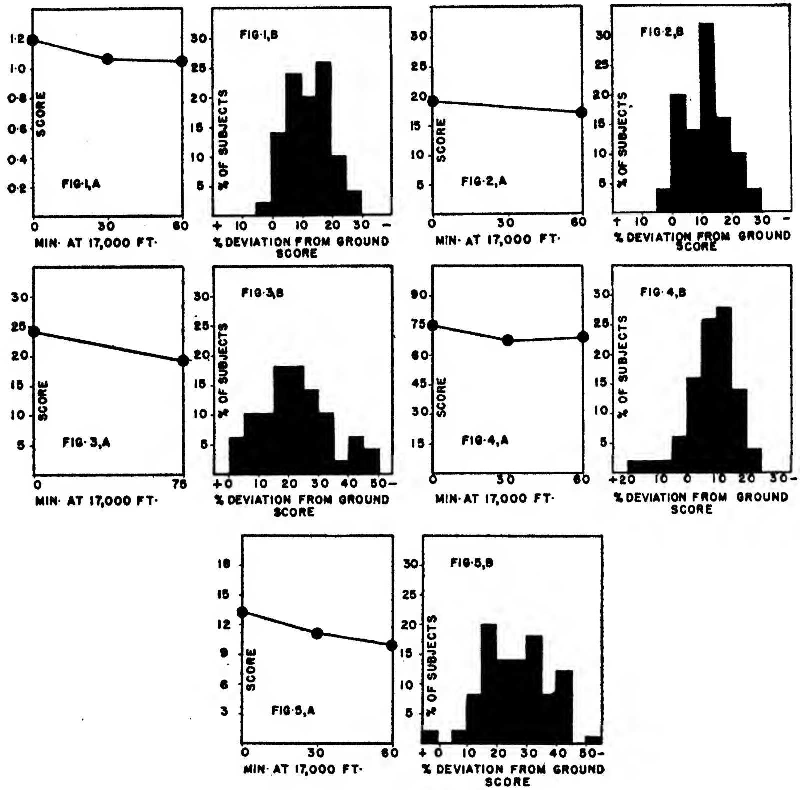
d_M: Per cent difference in mean test score decrements after 60–75 minutes at 17,000 feet (chamber altitude).

SEd_M: Standard error of the difference in means.
Differences in means are considered significant when $d_M/S.E.d_M$ exceeds 2.

in individual performance as judged by the average of all 5 test scores ranged from −1.6 to −28.6%, with a mean value of −15.7% (table 1). Relative susceptibility to anoxia as determined by mean percentage impairment correlated closely with that based on average rank (r = 0.947).

The degree of impairment in the average performance of the subject group as a whole on individual tests varied from a minimum of −7.8% in the cube-placing test to a maximum of −26.1% in the visual field perimetry test (table 1). The results obtained in each test manifested a reasonably high grade of internal

consistency when judged by the standard error of the mean (table 1) and the distribution curve of the scores (fig. 1–5).



FIGS. 1–5. EFFECTS OF ANOXIA

FIG. 1. Single dimensional pursuit-test performance. *a*. Average performance; *b*. variation in altitude effect.

FIG. 2. Vision demonstration card-test performance. *a*. Average performance; *b*. variation in altitude effect.

FIG. 3. Arithmetic test performance. *a*. Average performance; *b*. variation in altitude effect.

FIG. 4. Cube-placing test performance. *a*. Average performance; *b*. variation in altitude effect.

FIG. 5. Visual field area. *a*. Average performance; *b*. variation in altitude effect.

On analysis, the differences between the relatively small decrements in the cube placement, vision demonstration card, or single dimensional pursuit test scores and the proportionally large decrements in the arithmetic or visual field perimetry test scores proved to be significant (table 2).

No significant correlation was demonstrated between any two individual tests, nor did the degree of anoxic impairment in any single test correlate closely with that of the test battery as a whole. The arithmetic, single dimensional pursuit and visual field perimetry tests exhibited only fair correlation coefficients in this regard, while those of the cube-placement and visual-field perimetry tests were low (table 3).

A similar absence of intercorrelation has been reported by other observers for the effects of anoxia on perimetry, body sway and flicker fusion frequency (6).

TABLE 3. *The correlation of decrements in individual test scores with one another and with mean impairment in the test performance of the individual*

TEST	VISION DEMONSTRATION CARD	SINGLE DIMENSIONAL PURSUIT	ARITHMETIC	VISUAL FIELD PERIMETRY	AVERAGE (ALL TESTS)
Cube placing	−0.02	−0.07	0.00	−0.15	0.41
Vision demonstration card		−0.10	−0.01	−0.01	0.52
Single dimensional pursuit			0.09	−0.14	0.51
Arithmetic				−0.16	0.53
Visual field perimetry					0.18

r: Correlation coefficient.

CONCLUSIONS

The differences in percentage impairment of various aspects of group performance indicate that the quantitative changes observed under conditions of anoxia depend not only on the subject but also on the nature of the function under study. Net performance under such conditions is a composite result of alterations in a number of functions not similarly affected by an identical change in oxygen pressure. Such a result is not unexpected, since no a priori reason exists why a grade of anoxia sufficient to halve the ability to solve arithmetical problems necessarily should require a 50% reduction in manual dexterity or eye-hand coordination as well.

Individual subjects under anoxic conditions exhibit a considerable degree of variation both as to net decrement in performance and as to relative impairment of particular functions, whether these variations are estimated in terms of percentage differences or of rank differences. As a consequence, tests which measure a single function are not apt to be of selective value in singling out the individuals who can perform with maximum over-all effectiveness under low oxygen tensions.

The author wishes to express gratitude to the members of the staff of the Air Surgeon's Office and the School of Aviation Medicine whose assistance made this study possible.

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HISTAMINE CONTENT OF CANINE GASTRIC JUICE

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Received for publication September 18, 1947

A considerable body of direct and indirect evidence indicating that histamine plays a rôle in the secretory mechanism of the gastric mucosa has accumulated during recent years. Histamine has been demonstrated in the gastric mucosa of the hog (1), dog (2), cat (3) and man (4). Histaminase (diamine oxidase), though present in the intestinal mucosa, has been shown to be absent in the gastric mucosa of the dog (5). A histamine-like substance has been found in human gastric juice secreted spontaneously and in response to histamine (6, 7), and in gastric juice secreted by dogs in response to histamine, vagal stimulation and sham feeding (8, 9). A similar substance has also been demonstrated in gastric juice secreted by anesthetized cats or dogs in response to vagal stimulation, intraventricular injection of acetylcholine, intravenous injection of eserine, Uvnäs' gastrin (10), priscol and histamine, and in juice secreted in Pavlov pouches of dogs in response to a meal (3). Studies of the nature of this substance and of the circumstances in which it was found have been made in attempts to elucidate further the physiologic mechanisms involved in excitation of the secretory cells of the gastric mucosa. This investigation was undertaken to extend the results of others in a further search for possible correlations between the amounts of histamine present in gastric juice and the amounts of acid secreted by the gastric mucosa, and also to determine whether histamine was present in gastric juice secreted in response to stimulants which others had not tested, notably acetyl-beta-methylcholine chloride (mecholyl chloride) and insulin.

METHODS

Gastric juice was obtained from pouches of the stomach of 17 trained dogs. The pouches had been prepared with the animals under surgical anesthesia some weeks prior to utilization in the study. Eight of the animals had pouches of the Pavlov type, 7 had pouches of the Heidenhain type and 2 had transplanted gastric pouches. The gastric secretory stimulants employed were *a*) histamine given intramuscularly in a beeswax-sesame oil mixture prepared by the method of Code and Varco (11) and histamine given subcutaneously in saline solution, *b*) mecholyl chloride (acetyl-beta-methylcholine chloride, Merck) given subcutaneously and intravenously in saline solution, *c*) hypoglycemia induced by intravenous administration of regular insulin and *d*) a meal of ground raw horse meat taken by mouth. The animals were fasted for 18 to 24 hours before collections of gas-

[1] Present address: The University of Liverpool, England. Work done while on a fellowship from the Rockefeller Foundation.

tric juice were begun. In most tests samples of juice were collected for some hours during successive 30-minute periods.

The histamine equivalent of gastric juice was estimated by a modification of the procedures described by Barsoum and Gaddum (12) and Code (13). As a rule, extracts were prepared from 8 to 10 cc. samples of juice. When it was found that addition of trichloracetic acid to the juice did not bring down a precipitate, this step was omitted. The sample of juice was then boiled directly with 3 to 5 cc. of concentrated hydrochloric acid, dried *in vacuo* and an extract of the dried material prepared for assay on an isolated strip of guinea pig ileum suspended in 2.5 to 3.0 cc. of Tyrode's solution to which atropine sulfate (0.5 microgram per cubic centimeter) had been added.

Because of the small amounts of histamine often found in the gastric juice, the extracts were concentrated (2 volumes of juice to 1 volume of extract). Even then it was often necessary to add relatively large volumes of extract to the intestinal bath in order to induce a contraction; the maximal volume used at any time was 0.4 cc. It was soon found that when such volumes were used, accurate histamine assays were difficult. A substance or substances other than histamine apparently interfered with the determination. Potassium was suspected. Tests showed that solutions containing potassium in amounts similar to that in the concentrated extracts of gastric juice would produce contractions of the gut which could be mistaken for contractions due to small amounts of histamine. It was necessary, therefore, to reduce the potassium content of the extracts. This was done by insertion of an alcoholic extraction procedure like that recommended by Barsoum and Gaddum, except that much larger volumes of alcohol were used and no sodium chloride was added to the alcohol. The dried residue obtained after boiling and evaporating the juice was extracted three times with absolute alcohol, using 5 to 10 cc. for each extraction. The combined extracts were dried and the residue was dissolved in water or Tyrode's solution, then neutralized and made up to half the original volume of the juice. The extract was then ready for assay.

The efficiency of alcoholic extraction in removing potassium was tested by comparing the potassium content of extracts prepared with water, 90 to 95% alcohol and absolute alcohol (table 1, 14). We are grateful to Dr. M. H. Power for carrying out the potassium determinations by an adaptation of the method of Shohl and Bennett described by Hartzler (15). On the average the potassium content of the concentrated extracts was reduced from about 77 mgm. per 100 cc. when water was used to about 14 mgm. per 100 cc. when absolute alcohol was used (table 1). Up to 0.4 cc. of solutions containing the latter concentration of potassium could be added to the Tyrode's solution surrounding the strip of guinea-pig intestine without producing contractions. When it was also found that satisfactory recovery of histamine (79 to 81%) added to gastric juice was obtained by the alcoholic extraction procedure, it was then adopted as a routine.

With the procedure described histamine could be assayed quantitatively in concentrations of 5 or more micrograms per liter of gastric juice. Concentrations of 2.5 to 5 micrograms per liter could be detected but usually could not be determined quantitatively, and they were therefore recorded as 'trace'. With few

exceptions, the limit of histamine detectability by the method was 2.5 micrograms of histamine per liter. Since some histamine may have been present in extracts with equivalents below this value, use of the term 'zero' histamine content has been avoided and the designation 'undetectable' has been applied to such samples. All histamine assay values are expressed in terms of histamine base.

As a control to experiments in which gastric juice was obtained in response to the injection of mecholyl chloride, small quantities of the drug were added to samples of gastric juice and extracts prepared for histamine estimation. It was

TABLE 1. *Potassium content of dogs' gastric juice and extracts of gastric juice (all juice from Pavlov pouches and secreted in response to a meat meal)*

GASTRIC JUICE SAMPLE	MGM. POTASSIUM PER 100 CC.			
	Unextracted gastric juice	Gastric juice extracted and concentrated twice using		
		Water	90 to 95% alcohol	Absolute alcohol
1	45.7	87.7	33.0	15.3
2	43.2	81.6	36.0	13.7
3	37.6	76.1	30.6	14.0
4	46.4	81.5	29.4	12.8
5	39.8	66.0	43.8	13.6
6	43.0	73.9	36.8	14.2
7	28.0			12.6
8	36.1			15.4
9	32.8			13.4
10		77.4		13.2
11		75.3		13.5
12				15.3
13				10.9
Average......	39.2	77.4	34.9	13.7

Dogs' plasma 17.5 mgm. of potassium per 100 cc.
Tyrode's solution 10.5 mgm. of potassium per 100 cc.
Gastric juice secreted by dogs' total stomach pouches in response to histamine, 28.9 mgm. of potassium per 100 cc.

found that the addition of mecholyl chloride did not alter the histamine equivalent of the juice.

On completion of each series of experiments, a search was made for possible correlations between the histamine content of the juice and the acid content of the juice, and also between the histamine content of the juice and the rate of secretion of acid by the pouch.

RESULTS

(*a*) *Histamine equivalent of gastric juice secreted in response to the injection of histamine.* 1. Histamine in the beeswax mixture was administered intramuscularly in doses varying from 10 to 30 mgm. to 2 dogs with Pavlov pouches and 4 dogs with Heidenhain pouches. Thirty samples of gastric juice were tested.

The concentration of free hydrochloric acid in these varied between 0.412 and 0.620%. In 3 samples no histamine was detected; in 6, a trace was found. In the remaining 21 samples the histamine equivalents ranged from 5 to 80 micrograms per liter (table 2). The highest values occurred during the first hour after the injection of histamine.

2. Histamine in saline solution was administered subcutaneously in doses of 1.0 mgm. to 3 dogs with Pavlov type pouches, 3 dogs with Heidenhain pouches and 2 dogs with transplanted gastric pouches. One dog with a Pavlov type of pouch received only 0.5 mgm. of histamine. Twenty-eight samples of juice were studied. The concentration of hydrochloric acid of the majority ranged from 0.382 to 0.546%. In 2 samples, histamine activity was undetectable, in 4 samples a trace was present and in the remaining 22 the histamine equivalent varied from 5.0 to 36.4 micrograms per liter (table 3).

TABLE 2. *Histamine equivalent of dogs' gastric juice secreted in response to intramuscular injection of histamine in beeswax*[1]

ANIMAL	POUCH, TYPE	MICROGRAMS OF HISTAMINE EQUIVALENT PER LITER OF GASTRIC JUICE COLLECTED AT INTERVALS AFTER INJECTION OF HISTAMINE							
		½ hr.	1 hr.	1½ hrs.	2 hrs.	2½ hrs.	3 hrs.	3½ hrs.	24 hrs.
A	P	6.6	20.0	15.0		7.5			
B	P	33.3		20.8		25.0			
C	H	80.0		30.8		18.8	22.0		
D	H	57.2			33.3	13.7	6.6	6.6	
E	H	Tr	Tr	Tr	Tr	Tr	5.0		Tr
F	H	15.0	15.0	5.0	Un	5.0	Un		Un

[1] In this and subsequent tables H = Heidenhain pouch; P = Pavlov type of pouch; T = transplanted gastric pouch; Tr = trace of histamine detected in juice (2.5 to 5 micrograms of histamine per liter); Un = histamine undetectable in juice.

When the results of the two modes of histamine administration are combined, it is found that histamine activity was present in readily assayable quantities in 43 of the 58 samples of 'histamine' gastric juice. In an additional 10 samples it was present as a trace. In 5 samples it was undetectable. The conclusion is drawn that histamine is usually present in gastric juice secreted by Pavlov, Heidenhain and transplanted types of gastric pouches in response to the injection of histamine. It is noteworthy that histamine was undetectable by the methods employed in this study in 5 of the 58 samples tested.

There was no consistent indication of correlation between the concentration of histamine and the concentration of hydrochloric acid in the gastric juice when the histamine was given in either beeswax mixture or saline solution. Also, no correlation was evident between the concentration of histamine in the juice and

the output of hydrochloric acid per hour from the different pouches when histamine was given in saline solution. When the histamine was given in beeswax, the higher rates of acid output were associated with low concentrations of histamine activity, and the higher histamine equivalents were associated with low outputs of hydrochloric acid. For example, at rates of output of hydrochloric acid of more than 100 mgm. per hour, a histamine equivalent value of more than 15 micrograms per liter was present in only 2 of the 16 samples of such juice, while at rates of output of less than 100 mgm. of hydrochloric acid per hour histamine equivalents of more than 15 micrograms per liter were present in half the 14 samples. Thus, while half of the samples with comparatively low rates of acid output (less than 100 mgm. per hour) had low histamine activity, practically all of the samples with high rates of acid output had low histamine equivalents. Since even this correlation was absent when the histamine was given in saline solution, its existence is ascribed to the mode of administration of histamine.

TABLE 3. *Histamine equivalent of dogs' gastric juice secreted in response to subcutaneous injection of 1 mgm. of histamine base in physiologic saline solution*

ANIMAL	POUCH, TYPE	MICROGRAMS OF HISTAMINE EQUIVALENT PER LITER OF GASTRIC JUICE			
		Experiment number			
		1	2	3	4
A	P	6.3 5.0	Tr 5.3	Un	Un
B	P	36.4	36.4	21.4	35.8
C	H	15.6	7.5	31.3	7.5
F	H	5.0			
G	P	5.0	5.0	11.8	10.4
H[1]	P	14.3			
I	H	21.7	5.0	15.6	Tr Tr Tr
J	T	35.7			
K	T	30.0			

[1] 0.5 mgm. histamine injected subcutaneously in this animal.

(*b*) *Histamine equivalent of gastric juice secreted in response to the injection of mecholyl chloride*. As Gray and Ivy (16) have shown, when doses of mecholyl chloride which are optimal for stimulation of gastric secretion are given subcutaneously to dogs every 10 minutes, there occurs a peak and decline of gastric secretion over a period of about 4 hours. This sequence was reproduced in the

present experiments when mecholyl chloride was administered subcutaneously in doses of 0.1 to 0.15 mgm. every 10 minutes and intravenously by continuous drip in doses of 0.5 to 1.0 mgm. per hour in 5 dogs that had Heidenhain pouches.

1. In one group of experiments the histamine equivalents of individual samples of gastric juice secreted in response to injection of mecholyl chloride were estimated. Histamine activity was present in readily estimable quantities in all of the 18 samples obtained. The concentration of activity varied from 7.2 to 140.0 micrograms per liter (table 4). In three experiments in which sufficient juice was secreted for assay of consecutive samples, high values occurred during the initial period of stimulation (table 4).

TABLE 4. *Histamine equivalent of gastric juice secreted by Heidenhain pouches in dogs in response to subcutaneous or intravenous injection of mecholyl chloride*

ANIMAL	MICROGRAMS OF HISTAMINE EQUIVALENT PER LITER GASTRIC JUICE					
	Experiment number					
	1	2	3	4	5	6
C	26.3[1]	25.5[1]	25.0[1]	17.9[1]	7.2[1]	25.0[1]
F	45.8[1]					
I	140.0[1]	117.5[2] 57.2	22.2[1]	41.7[2] 27.8 25.0 35.0 41.7		
L	83.3[2] 31.2					

[1] Pooled samples of entire experiment.
[2] Consecutive samples collected during 60- to 90-minute periods.

Although in one experiment there was a tendency for the higher histamine equivalents to be associated with the lower concentrations and lower outputs of hydrochloric acid, in general there was no correlation between the concentration of histamine activity in the juice and the concentration of hydrochloric acid or the rate of output of hydrochloric acid from the mucosa.

2. A second group of experiments was carried out to test further whether there was a correlation between the rate of secretion of hydrochloric acid and the histamine equivalent of the gastric juice. Mecholyl chloride was given subcutaneously every 10 minutes on nine different occasions to each of 3 dogs with Heidenhain pouches. Samples of gastric juice from each of the 3 dogs were collected and accumulated separately according to their rates of secretion of hydrochloric acid. The secretory rates selected for separate collections were 0–20, 21–40, 41–60 and so on up to 181–200 mgm. of hydrochloric acid per hour.

All of the pooled samples contained histamine in readily estimable quantities (8–40 micrograms per liter). There was no positive correlation between the rate

of secretion of hydrochloric acid and the amount of histamine activity in the juice. Indeed, the highest histamine equivalent values were consistently associated with the lower rates of acid secretion (table 5).

(c) *Histamine equivalent of dogs' gastric juice secreted in response to hypoglycemia.* Hypoglycemia was induced in experiments on 4 dogs with Pavlov

TABLE 5. *Histamine equivalent of gastric juice secreted by three dogs with Heidenhain pouches in response to mecholyl chloride*

RATE OF SECRETION OF HYDROCHLORIC ACID, MGM. PER HR.	MICROGRAMS HISTAMINE ACTIVITY PER LITER GASTRIC JUICE		
	Animal		
	M	C	F
0–20	27.0 (9)[1]	19.0 (8)	16.0 (5)
21–40	40.0 (8)	18.0 (6)	23.0 (7)
41–60	21.0 (7)	15.0 (5)	20.0 (4)
61–80	23.0 (3)	10.0 (1)	10.0 (7)
81–100	9.0 (1)		8.0 (2)
101–120			12.0 (4)
121–140			12.5 (1)
141–160		8.0 (1)	12.5 (1)
181–200		8.0 (1)	

[1] Values in parentheses are the number of separate samples of juice comprising the pooled sample on which the histamine equivalent was determined.

types of pouches by the intravenous injection of from 0.5 to 1.0 unit of regular insulin per kilogram of body weight. Among 11 samples of resulting gastric juice, estimable quantities of histamine varying in concentration from 6.3 to 25.0 micrograms per liter were present in 7, a trace was found in one and no histamine was detectable in 3. Concentration of free hydrochloric acid in the juice varied from 0.197 to 0.409%. The conclusion is drawn that histamine activity is usually present in gastric juice secreted by the dog in response to hypoglycemia.

The number of tests in this series of experiments has been regarded as too small

to give a satisfactory appraisal of possible histamine-acid relationships. The data obtained, however, gave no evidence of a consistent relationship between the concentration of histamine activity and the concentration or output of hydrochloric acid in the juice.

TABLE 6. *Histamine equivalent of gastric juice secreted by dogs with Pavlov-type pouches in response to feeding of 150 to 300 grams of raw horse meat*

<table>
<tr><th rowspan="2">ANIMAL</th><th rowspan="2">TEST</th><th colspan="8">MICROGRAMS HISTAMINE BASE PER LITER GASTRIC JUICE SECRETED AFTER MEAT MEAL</th></tr>
<tr><th>½ hr.</th><th>1 hr.</th><th>1½ hr.</th><th>2 hr.</th><th>2½ hr.</th><th>3 hr.</th><th>3½ hr.</th><th>4 hr.</th></tr>
<tr><td>A</td><td>1</td><td>15.0</td><td>5.0</td><td>Tr</td><td>Un</td><td>Tr</td><td></td><td></td><td></td></tr>
<tr><td></td><td>2</td><td>Un</td><td>Un</td><td>Un</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>3</td><td>12.0</td><td>5.0</td><td>5.0</td><td>5.0</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>4</td><td>11.2</td><td>Tr</td><td>Tr</td><td>Tr</td><td colspan="2">Tr</td><td colspan="2">Tr</td></tr>
<tr><td></td><td>5</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td></td></tr>
<tr><td></td><td>6</td><td>6.6</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td></td></tr>
<tr><td></td><td>7</td><td>Tr</td><td>Tr</td><td>Tr</td><td>Tr</td><td>Tr</td><td>Tr</td><td>Tr</td><td></td></tr>
<tr><td></td><td>8</td><td>Un</td><td></td><td>Tr</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td></td></tr>
<tr><td></td><td>9</td><td>8.5</td><td>6.3</td><td>Tr</td><td>Tr</td><td>Tr</td><td>5.0</td><td></td><td></td></tr>
<tr><td>B</td><td>1</td><td>37.5</td><td></td><td colspan="3">16.6</td><td colspan="3">13.6</td></tr>
<tr><td></td><td>2</td><td>25.0</td><td>7.9</td><td colspan="2">8.6</td><td colspan="2">9.3</td><td colspan="2">8.4</td></tr>
<tr><td>G</td><td>1</td><td>6.6</td><td>6.6</td><td>Tr</td><td>Tr</td><td>Tr</td><td>Tr</td><td></td><td></td></tr>
<tr><td></td><td>2</td><td>5.9</td><td>5.9</td><td>5.0</td><td>Tr</td><td>Tr</td><td>Tr</td><td></td><td></td></tr>
<tr><td></td><td>3</td><td>Tr</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td>Un</td><td></td></tr>
<tr><td>H</td><td>1</td><td>25.0</td><td>12.5</td><td>15.0</td><td>15.0</td><td>11.3</td><td></td><td></td><td></td></tr>
<tr><td></td><td>2</td><td>8.0</td><td>7.0</td><td>5.0</td><td>Tr</td><td>Tr</td><td>5.0</td><td>Tr</td><td></td></tr>
<tr><td></td><td>3</td><td>26.7</td><td>6.3</td><td>Tr</td><td></td><td>Tr</td><td></td><td></td><td></td></tr>
<tr><td></td><td>4</td><td>58.8</td><td>14.3</td><td>10.0</td><td>8.0</td><td></td><td></td><td></td><td></td></tr>
<tr><td>N</td><td>1</td><td>9.0</td><td>22.0</td><td colspan="2">5.0</td><td colspan="2">7.5</td><td colspan="2">12.0</td></tr>
<tr><td>O</td><td>1</td><td>Tr</td><td>12.0</td><td>5.6</td><td>6.0</td><td>5.0</td><td>5.0</td><td></td><td></td></tr>
<tr><td>P</td><td>1</td><td>40.0</td><td>20.0</td><td>18.2</td><td>22.2</td><td>21.1</td><td>27.0</td><td></td><td></td></tr>
<tr><td></td><td>2</td><td>Un</td><td></td><td>Un</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>3</td><td>35.0</td><td colspan="2">30.0</td><td>22.5</td><td>20.0</td><td></td><td></td><td></td></tr>
<tr><td>Q</td><td>1</td><td>Un</td><td>Tr</td><td colspan="3">Tr</td><td></td><td></td><td></td></tr>
</table>

(*d*) *Histamine equivalent of dogs' gastric juice secreted by Pavlov pouches in response to a meat meal.* Histamine estimations were made on 124 samples of gastric juice secreted from Pavlov types of pouches in 8 dogs during 24 different tests in which the animals ate a meat meal. Histamine concentrations of 5.0 to 58.8 micrograms per liter were found in 59 of the 124 samples. Traces of activity were present in 34 samples, and no activity was detectable in the remaining 31 samples (table 6).

In the 17 experiments in which activity was present in sufficient concentration

for a quantitative assay, the histamine equivalent of the juice secreted during the first hour after feeding was higher than that secreted subsequently (table 6). In an effort to determine whether these initial high values were due to a washing out of histamine lying in the resting gastric glands, experiments were performed in which, to flush out the glands, a subcutaneous injection of 0.5 to 1.0 mgm. of histamine was given 45 to 90 minutes before feeding the meal. In 4 of 5 tests in which estimable quantities of histamine activity were present, the gradient of greater to lesser histamine equivalents during the progress of the response to the meal was preserved even after the preliminary washing out of the gastric glands by the juice secreted in response to the histamine (table 7).

Thus, when estimable quantities of activity were present, there was a definite sequence of changes in the amount of histamine activity in the juice as the response to the meal progressed. This sequence has been compared with the sequence of changes in concentration of hydrochloric acid in the juice and output of hydrochloric acid from the pouch. In 21 of the 24 experiments maximal output of hydrochloric acid (milligrams of hydrochloric acid per hour) was reached in the second half-hour period when the acid concentration had also reached or very nearly reached its maximum, but with few exceptions the amount of histamine activity in the juice had by then definitely declined. During the remainder of the response the concentration of histamine activity leveled off at low values, the acid concentration leveled off at a high value showing only minor fluctuations about its maximum and the acid output, although declining gradually, still continued at a high rate. This sequence of changes is illustrated graphically in figure 1. A representative example of the variability between tests in one animal is shown (left side of figure 1), and the close similarity in the pattern of the changes in different dogs with similar pouches is illustrated by the averages of a number of tests on each of three animals (right side of figure 1).

TABLE 7. *Histamine equivalent, micrograms per liter, of gastric juice from Pavlov pouches in dogs secreted in response to subcutaneous injection of 0.5 to 1.0 mgm. of histamine followed by a meat meal*

ANIMAL	TEST	HOURS BEFORE MEAL	HOURS AFTER MEAL								
		¾	½	1	1½	2	2½	3	3½	4	4½
A	11	Un	Un	Un	Un		Un				
	12	Un	Un	Un	Un	Un	Un	Un	Un	Un	
B	3	[1]	19.1	8.3	5.0	10.0		8.3		8.3	
	4	35.8	18.5		18.5				18.0		
G	4	11.8	5.6	5.0	Tr	Tr	Tr				
	5	10.4	6.2	Tr	Tr		Tr		Tr		
H	5	14.3	14.3	7.5	6.3	5.0	6.5	7.0	7.3	6.5	

[1] Histamine equivalent not determined.

As expected from the sequence illustrated, there was a tendency for the higher histamine equivalents to be associated with the lower concentrations of hydrochloric acid and for the lower histamine equivalents to be found in the juice with the higher acid concentrations. Complete data were obtained during 18 trials with 6 of the animals. One of the animals consistently showed so little histamine in the juice that an accurate search for correlations could not be made. In the remaining 5 dogs, on which 14 tests were made, histamine activity was present in amounts which allowed quantitative assay in 12 instances. In all but two of these the highest histamine activity values were associated with the lowest con-

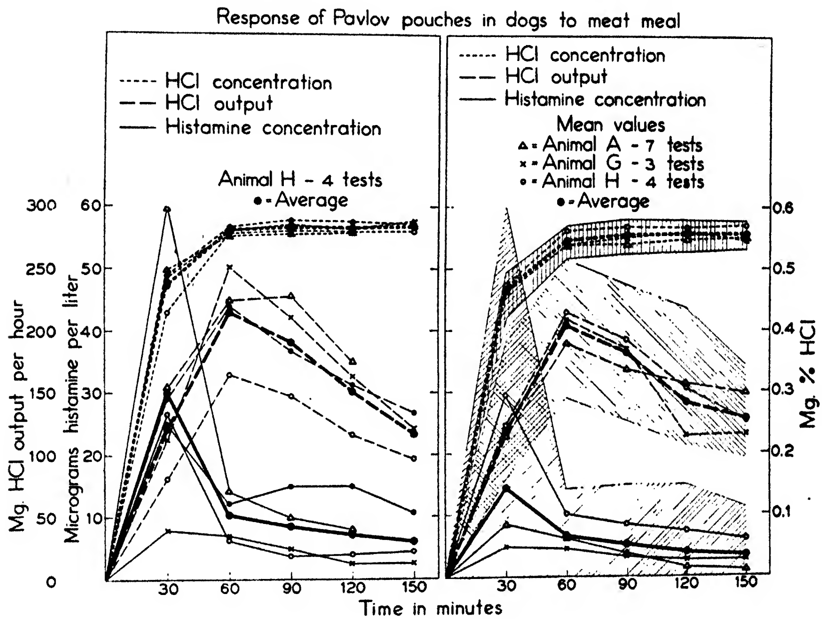

FIG. 1. CONCENTRATION OF HYDROCHLORIC ACID, OUTPUT OF HYDROCHLORIC ACID AND HISTAMINE ACTIVITY IN GASTRIC JUICE secreted during 30-minute periods from Pavlov types of gastric pouches in dogs after eating a meat meal.

centrations of hydrochloric acid and occurred in the first sample of juice secreted. Also, in every experiment the first sample of juice collected had the lowest acid concentration. With the exception of only one test, the high concentrations of hydrochloric acid were found in juice containing small or inestimable quantities of histamine activity.

There was not a direct correlation between the concentration of histamine activity and the output of hydrochloric acid per hour by the pouches. As a rule, however, maximal acid outputs from the pouches were accompanied by low or inestimable concentrations of histamine activity.

Two points of physiologic significance derived from this search for correlations

deserve emphasis. First, the highest concentration of histamine activity usually occurred in the juice secreted during the first 30 minutes, when the concentration of acid in the juice was lowest and the output of hydrochloric acid was just building up to a maximum. Later in the response the histamine concentration in the juice consistently fell to a low level, while the acid concentration stayed at or near maximum and the output of hydrochloric acid declined only slowly. In these experiments, therefore, high histamine equivalents were associated with the initiation of secretion, and low equivalents were associated with the continuation or maintenance of secretion. Second, in the later stages of the response to the meal, as in the case of the response to the injection of histamine, a very efficient secretion of hydrochloric acid consistently occurred in conjunction with very low concentrations of histamine activity in the juice.

COMMENT

In this study the terms 'histamine activity' and 'histamine equivalent' have been used because it is felt that until the active constituent in the extracts of gastric juice has been chemically isolated and identified, its definite designation cannot be made. Experience gained in the research, however, supports the view that the active ingredient in the final extracts was histamine.

When histamine was given in beeswax, the high concentrations of activity in the juice always occurred early in the response. Previous experience has shown that this is usually the time of maximal absorption of histamine from waxy mixtures. Particles of histamine located at the surface of the injected mass are apparently quickly dissolved and absorbed. Sometimes, with large doses, sufficient histamine has been liberated at this time to produce symptoms of shock, and in the present study this did occur on one occasion. In the presence of such toxic concentrations gastric secretion is often slight or absent. Thus, just as gastric secretion was starting, the maximal amount of histamine was circulating in the blood and this quantity may often have been in excess of that needed for optimal stimulation of gastric secretion. It is probable that these factors account for the association of high concentrations of histamine activity with low rates of acid output in the juice secreted during the initial phases of the response to histamine in the beeswax mixture.

In the study, 58 samples of gastric juice secreted in response to the injection of histamine were tested. In 5, histamine activity was absent or at least undetectable. The finding is important since there can be little doubt that these samples of juice were produced as a result of the action of histamine in the gastric mucosa.

Also, while in general, with histamine stimulation, correlations between the amounts of histamine activity and content and output of hydrochloric acid in the juice were lacking, one fact of physiologic importance is evident. High concentrations and rapid rates of output of hydrochloric acid often occurred when the concentration of histamine activity in the juice was exceedingly low or even undetectable by the methods used in the study. Thus, even when the gastric mucosa was being stimulated by histamine brought to it by the blood stream, maximal concentrations and high rates of output of hydrochloric acid occurred in the juice

without the cause of the stimulation, in this case histamine, being detectable in the juice. In view of this result, when histamine activity was undetectable in samples of juice resulting from other forms of stimulation, the conclusion could not then be drawn that the juice had been produced without the participation of histamine in the secretory process.

In experiments in which gastric juice was collected from the whole stomach during vagal stimulation, MacIntosh found no correlation between the acidity or digestive power of such juice and its histamine equivalent. He did note, however, an apparent tendency for a high rate of secretion to be associated with a high histamine equivalent. Such a result was not obtained in the present investigation. It should be emphasized, however, that direct vagal stimulation was not employed in our study, and juice was obtained from pouches of the stomach and not from the entire stomach. These differences may account for the differences in the results. They serve also to emphasize one of the limitations of the present investigation.

Not all of the factors which produce secretion in the stomach of the intact animal as the result of eating a meal were tested in the experiments reported. Psychic (nervous) and humoral mechanisms were most likely responsible for the secretion from the Pavlov pouches following the ingestion of the meal, but in the intact stomach the presence of the meal itself produces local stimulation and the histamine equivalent of juice so derived was not determined in this study.

The general lack of positive correlations between the concentration of histamine activity and the amount or rate of secretion of hydrochloric acid observed in our experiments might, on first consideration, be regarded as opening to some question the validity of the hypothesis that histamine is concerned in the mechanism which produces acid gastric juice. The possibility that the presence of histamine activity in gastric juice is purely incidental to the general metabolism of the mucosal cells and is not related directly to the secretion of gastric juice must be considered in the light of these experiments. Yet so much evidence for the participation of histamine or a histamine-like substance in gastric secretion has accumulated that application of the histamine hypothesis seems more justified and more likely to be productive than if some other interpretation is sought. From this viewpoint the lack of correlation may be taken to indicate that not all of the histamine or not all of the secretory factors concerned were being determined by simply estimating the amount of histamine activity in the juice. The cells of the gastric mucosa may offer a barrier to the passage of histamine into the juice, and the amount allowed to pass into the juice may vary under different circumstances. Also, the amounts of histamine which must be free in the mucosa to initiate secretion, and the amounts which are needed to maintain secretion, may differ and may vary with changing conditions. In addition, the demonstration by Brunschwig and co-workers (17, 18) of a gastric secretory inhibitor in gastric juice indicates that other factors may also be involved. If secretory and inhibitory substances are normally in operation, then correlations will certainly not be obtained until both can be measured quantitatively and simultaneously.

In the light of such considerations it seems evident that little more of a definite

nature can be stated as a result of the experiments reported here than that histamine activity was usually present in canine gastric juice secreted from pouches in response to a variety of stimuli and that the data are in general agreement with the concept that histamine may be a common local chemostimulator to the cells of the gastric mucosa for a variety of physiologic mechanisms which lead to the production of gastric juice.

SUMMARY

1. Gastric juice secreted in response to the injection of histamine as a rule manifested histamine activity. In some samples, however, none was detectable by the methods used in this study.
2. Gastric juice secreted in response to mecholyl chloride uniformly contained readily estimable quantities of histamine activity.
3. Gastric juice secreted in response to the intravenous injection of insulin in doses of 0.5 to 1 unit per kilogram of body weight usually manifested histamine activity.
4. Three fourths of the samples of gastric juice secreted from Pavlov pouches in response to the feeding of a meat meal contained detectable quantities of histamine activity. When present in estimable quantities, the concentration of histamine activity in the juice was greatest during the first hour of the response to the meal.
5. In general, high concentrations of histamine activity were found in juice in which the concentration of hydrochloric acid was comparatively low and the acid output from the pouches (milligrams of hydrochloric acid per hour) was also low; high concentrations of acid in the juice and high acid outputs from the pouches were as a rule associated with low concentrations of histamine activity.

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ROLE OF THE KIDNEYS IN MODIFYING THE RESPONSE TO THE 'SUSTAINED PRESSOR PRINCIPLE'

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Received for publication October 7, 1947

In another communication (1) it was reported that blood plasma obtained from cats which had been subjected to ½–2 hours of hypotension was capable of causing a sustained elevation of blood pressure (BP) when injected intravenously into cats that had been nephrectomized two days prior to the tests. The unusual pressor effect was believed to be due to the presence in the plasma of a previously undescribed pressor principle which had emanated from the kidneys during the period of hypotension. The intravenous injection of plasma containing the pressor principle into normal non-nephrectomized cats was observed to cause only unsustained elevations of BP. As a possible explanation for the difference in responses, it was suggested that the kidneys might be concerned in the elaboration of a substance which could inactivate the pressor principle or otherwise inhibit its action.

In the present paper further observations are reported on the possible rôle played by the kidneys in modifying the responses to the pressor agent.

METHODS

Active plasmas, i.e., those capable of causing a sustained elevation of pressure, were obtained from the terminal blood of cats which had died of 'natural causes' or from DDT poisoning, or from cats which had been subjected to a period of hypotension as the result of bleeding (1). Later it was found that plasma containing the pressor substance could also be obtained from the blood of cats which had been given intravenous injections of semicrude extracts of cat kidneys (2).

A satisfactory method for standardizing the sustained pressor activity is not yet available. Although the pressor potency of different plasmas varies somewhat, 2 ml. has been arbitrarily chosen as the quantity of plasma to be injected, so that a relative, if inexact, comparison of the pressor responses could be made. In all instances in which consecutive injections were made, the same plasma was used throughout the experiment.

Two different types of test animals were prepared: *a*) normal cats were nephrectomized under pentobarbital sodium anesthesia, and from 1 to 48 hours later they were pithed[1] as previously described (1); *b*) normal cats were anesthe-

[1] Pithed preparations were used exclusively in the present experiments for several reasons: *a*) the blood-pressure level in the pithed cat is relatively stable; *b*) the necessity for maintaining a constant level of anesthesia is eliminated; and *c*) because of the normally low blood pressure, three or four consecutive sustained pressor responses may be obtained

tized and through a midline incision each kidney was freed from the surrounding fat and adjacent peritoneum. A loop of heavy thread was placed loosely around each renal pedicle and the ends carried to the outside through the operative incision. Eighteen to 24 hours later, *a*) before anesthesia, *b*) after anesthesia but before pithing or *c*) after pithing, the ends of each thread were passed through a small tube which was then inserted through the incision to the region of the renal pedicle. By applying traction on the threads the renal pedicles were 'snared' tightly against the ends of the tubes. It was felt that by this procedure the animal would be functionally 'nephrectomized' with the least possible trauma. The effectiveness of the clamping procedure was verified at the end of each experiment by administering a dye intravenously and examining sections of the kidneys postmortem.

RESULTS

Including the experiments previously reported concerning the sustained pressor principle (1, 2), plasmas from the terminal bloods obtained from 123 cats have possessed the ability to cause a sustained elevation of BP when injected intravenously into pithed cats which had previously been bilaterally nephrectomized. The active plasmas were obtained from 12 cats which had died spontaneously, 60 which had been killed by the intraperitoneal injection of a solution of DDT, 13 which had been subjected to hemorrhagic hypotension for a period of 30 minutes–2 hours or from 38 which had received intravenous injections of semicrude extracts of cat kidneys.

Among the different types of test animals the responses to the active plasmas varied in several ways. There was a fair degree of correlation between the time following nephrectomy and the observation of a sustained pressor response to the initial injection. In general the cats nephrectomized two days before were quite sensitive and seldom failed to exhibit sustained responses. However, the animals which had been nephrectomized only a few hours before often responded to the injection of active plasma with a rise in BP which subsided to the base level in 10–20 minutes. Repeated injections of the same plasma were sometimes followed by similar unsustained responses, but frequently the second or third injection caused well sustained pressure elevations. In table 1 are presented the results of all cat experiments performed to date, tabulated according to the type of response obtained with the eight different kinds of preparations used.

DISCUSSION

Repeated injections of active plasma into normal cats with kidneys intact have not been found to cause a sustained elevation of BP (table 1, column 5); the typical response observed was an acute rise in pressure (15–30 mm. Hg) which subsided completely over a period of 10–20 minutes. If the return of

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before the animal's maximum blood-pressure level is reached. The injections were begun when the BP had reached a reasonably constant level, which was usually 20–40 minutes after pithing.

the BP to the control level is interpreted to mean that the sustained pressor principle was being inactivated or destroyed, the most likely possibilities to be considered are mechanisms for inactivation within the kidney or a substance elaborated by the kidney. The fact that unsustained responses have been obtained up to 48 hours after the kidneys had been removed (table 1, line 2, columns 1, 2, 3, 4) indicates that any inactivating mechanism involving the actual presence of the kidneys in the animal cannot be the only factor responsible for the subsidence of the pressor effect.

	COLUMN	1	2	3	4	5	6	7	8
		PREVIOUSLY NEPHRECTOMIZED				NOR. NON-NEPH.	RENAL PEDICLES CLAMPED		
	HOURS	48	24	6	1		BEFORE ANESTHESIA	BEFORE PITHING	AFTER PITHING
LINE	CAT PREPARATIONS-TOTAL	197	33	14	12	10	6	6	9
1	INITIAL PRESSOR RESPONSES SUSTAINED	155	22	6	2 N-S(2.0) N-S(1.75)	0	0	2 C-S(1.7) C-S(2.0)	0
2	INITIAL RESPONSES UNSUSTAINED. SUBSEQUENT RESPONSES SUSTAINED.	1	6	6	1 N-3U-S(1.5)	0	5 C-4U(1)S(1.5) C-3U(1)S(1.7) C-3U(1)S(2.0) C-2U(1.7)S(2.0) C-2U(1.7)S(2.2)	2 C-3U(2.0)S(3.2) C-2U(0.6)S(1.0)	5 3U-C-1U-S(0.5) 3U-C-2U-S(1.2) 3U-C-3U-S(1.0) 3U-C-3U-S(1.0) 1U-C-1U-S(0.5)
3	ALL RESPONSES UNSUSTAINED: VERY LOW B.P <40 MM. HG.	30	5	1	5	1	1	0	0
4	ALL RESPONSES UNSUSTAINED: CARDIAC IRREG., HEMORRHAGE, PULM. EDEMA, PNEUMONIA	4	0	0	0	0	0	0	1
5	ALL RESPONSES UNSUSTAINED: ANIMAL APPARENTLY IN GOOD CONDITION (SEE TEXT)	7	0	1	4 N-3U(3-4) N-2U(3-4) N-10U(1-4) N-3U(0.7-2.5)	9	0	2 C-6U(1.5-4.5) C-5U(0.7-3.5)	3 3U-C-6U(2.5) 3U-C-5U(2.0) 4U-C-4U(2.2)
6	AVE. BUN-MGM/100ML. (STARVED 24-48 HRS.)	173	92	46	32	28	36		

TABLE 1

Abbreviated notations in columns 4, 6, 7 and 8 indicate sequence of procedures and observations. *N*, nephrectomy; *C*, renal pedicles clamped; *U*, unsustained responses; and *S*, sustained responses. Unbracketed numerals preceding *U* or *S* indicate number of responses observed. Bracketed numerals indicate number of hours following nephrectomy or clamping that the responses were observed. Example—(1st experiment, column 6, line 2) pedicles clamped, 4 unsustained responses obtained within one hour after clamping, first sustained response observed 1.5 hours after clamping.

Mean blood pressure tracings of pithed cats. Ordinate scales—mm. Hg. Time marker —1 minute.

In many instances one or more unsustained responses were obtained, following which subsequent injections of the same plasma caused sustained pressor responses (table 1, line 2; figs. 1 and 2). In the nephrectomized series this phenomenon occurred most frequently in the cats from which the kidneys had been removed 1–6 hours previously, and it was not uncommon in the 24-hour group. Unsustained initial responses were also observed in the animals in which the kidneys were excluded from the circulation by constriction of the renal pedicles (table 1, line 2, columns 6, 7, 8; fig. 3). Although the evidence available at the present time is not conclusive, the experiments suggest the possibility that an inhibitor of the pressor substance could have remained in the nephrectomized animals and accounted for the subsidence of the BP elevations; with repeated injections of the pressor material the hypothetical inhibitor may have been 'neutralized' or exhausted, and thereafter the pressor responses were sustained.

If such an inhibitor mechanism or substance does remain in the animal after removal of the kidneys, its effectiveness is relatively short-lived when repeated injections of active plasma are given. Even without injections of the pressor material there appears to be a spontaneous disappearance of the inhibitor effect, since about one-half of the test cats nephrectomized 6 hours before, three-quarters of the 'one-day nephrectomized' cats and essentially all of the 'two-day nephrectomized' cats exhibited initial responses which were sustained (table 1, line 1, columns 1, 2, 3). As the length of time after nephrectomy increases,

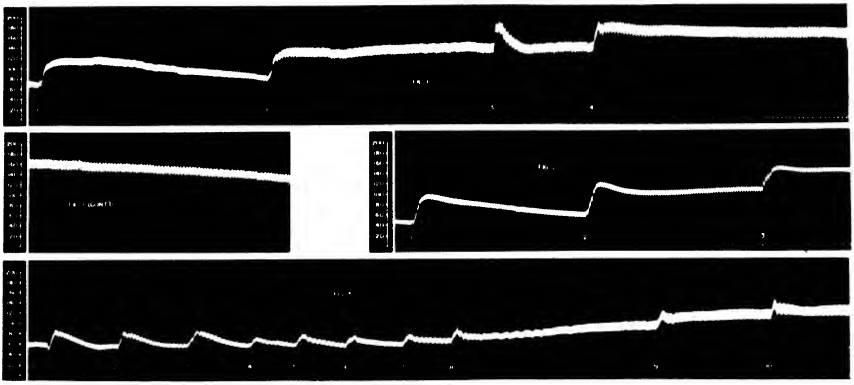

FIG. 1. CAT NEPHRECTOMIZED 24 HOURS BEFORE. Initial IV injection of 2 ml. of dialyzed active cat plasma at *1* caused unsustained pressor response. Injection of 2 ml. of the same plasma at *2* and *4* caused sustained responses; *3*, injection of angiotonin.

FIG. 1 (*cont'd*). Remainder of record showing spontaneous gradual decline of BP toward end of experiment (see text) (32ZC-367).

FIG. 2. CAT NEPHRECTOMIZED 6 HOURS BEFORE; 2 ml. of same active plasma injected at *1*, *2* and *3*. Unsustained initial pressor response followed by two sustained responses (32ZC-315).

FIG. 3. NORMAL CAT with loops of thread placed (without constriction) around renal pedicles 24 hours before. Two milliliters of dialyzed, active cat plasma injected at *1*, *2*, *3*, *5*, *6*, *7*, *8*, *9*, *10*. Threads tightened around both renal pedicles at *4*. Line drawn at mean BP level of 50 mm. to facilitate visualizing sustained elevation of pressure (32ZC-376).

the animal is progressively more likely to maintain the elevation of BP following the first injection of active plasma.

In many of the experiments it was not possible to demonstrate sustained rises in BP even with repeated injections of plasma given over several hours. The physiological state of the animal late in the experiment is a factor which may be responsible for the failure to observe sustained responses in certain of the experiments (see table 1, line 5). There was usually evident, from 2 to 5 hours after pithing (and occasionally sooner), a very gradual, spontaneous decline in the cats' BP, which was more noticeable if the BP had previously been elevated by the injection of active plasma (see fig. 1, *cont'd.*); thereafter, injections of plasma caused comparable elevations of BP, but they were invariably unsustained. Under these circumstances it was not possible to tell

whether a specific inhibitor mechanism was still functioning or whether the general condition of the animal had deteriorated to such an extent that the induced elevation of BP could not be maintained.

The degree of azotemia (table 1, line 6) and the retention of other substances associated with impending uremia are probably not concerned in determining the *type* of response to the pressor principle, since unsustained and sustained responses are observed to occur consecutively within a period of 20–30 minutes, an interval which would seem much too short for any significant 'uremic' change to have taken place. Furthermore, sustained responses have been obtained within an hour after constriction of the renal pedicles, and at the end of these experiments the blood urea nitrogen was found to be slightly elevated but not greatly in excess of the average normal level. Conversely, unsustained initial responses were observed in animals nephrectomized 24 hours before, although the degree of nitrogen retention was considerably greater (table 1, line 6).

However, there are indications that as the period of time following nephrectomy increases, the animals' *sensitivity* to the pressor principle also increases. The sustained elevations in pressure were often 1.5 to 4 times as great in the pithed cats, nephrectomized two days before, as in those nephrectomized only a few hours before. Also, the maximum level to which the BP could be raised by repeated injections of active plasma usually ranged between 160 and 180 mm. Hg in the 'two-day nephrectomized' pithed cats, while the top levels for the recently nephrectomized animals more often varied between 110 and 150 mm. Hg. The explanation for the progressive increase in sensitivity is not known. It has been a common finding that cats which have been nephrectomized one or two days prior to testing are more sensitive to angiotonin and also to tyramine. It is conceivable that some nonspecific change in the animal incident to the development of uremia could be responsible for augmenting the responses to these substances as well as to the sustained pressor principle.

Last to be considered is the rôle the kidneys may play directly in preventing or abolishing the sustained pressor effect of the pressor substance. Although none of the animals with intact kidneys have exhibited sustained responses at any time, it has not yet been determined to what extent, if any, the pressor principle may have been 'inactivated' during passage through the kidneys. The unsustained responses obtained before and immediately after clamping of the renal pedicles were not materially different in magnitude or contour (fig. 3), and under these conditions the exclusion of the kidneys from the circulation did not immediately lessen by a discernible amount the existing tendency for the responses to be unsustained. The apparent inactivation or destruction of the pressor principle, therefore, could not be attributed exclusively to an intrarenal mechanism. Since unsustained pressor responses were observed to occur progressively less frequently as the interval of time following nephrectomy was increased, and since sustained responses were often obtained only after repeated injections of active plasma (table 1, line 2), it would appear more likely that: *a*) the kidneys may secrete a substance which is an inhibitor or otherwise functions as an antagonist to the sustained pressor principle; *b*) the hypothe-

tical inhibitor spontaneously disappears from the animal following bilateral nephrectomy; *c*) it is apparently 'neutralized' or rendered ineffective by the repeated intravenous administration of plasma containing the pressor principle.

SUMMARY

As previously reported there appears in the blood plasma of cats a pressor substance which presumably emanates from the kidneys as a consequence of decreased blood flow (or blood pressure) within these organs. Unlike other known biological pressor substances, it possesses the ability to cause a sustained elevation of BP when injected intravenously into cats which have been nephrectomized several hours to several days before.

Repeated injections of plasma containing the pressor principle have caused only acute, unsustained rises in blood pressure in normal cats (kidneys intact). In animals from which the kidneys have been acutely removed or excluded from the circulation, the initial pressor responses were unsustained, but with repeated injections of the plasma sustained responses were ultimately observed.

Cats which had been nephrectomized 6–24 hours before not infrequently exhibited unsustained responses to the first injection of plasma containing the pressor principle, but the responses to the second or third injections were well sustained. The initial responses in cats nephrectomized 48 hours before were almost invariably sustained.

The failure of certain of the pressor responses to be sustained suggests the possibility that the pressor principle injected into the blood stream may be inactivated or its action otherwise inhibited. In the non-nephrectomized animals it has not yet been determined to what extent, if any, 'inactivation' may have taken place within the kidneys. Other experiments in the present study offer supporting evidence for the hypothesis that: *a*) the kidneys may secrete an inhibitor of the pressor principle under conditions of normal blood flow (or blood pressure) within these organs; *b*) the inhibitor remains in the animal after removal of the kidneys but diminishes progressively as the time following nephrectomy increases; and *c*) the inhibitor may be neutralized or exhausted by repeated injections of plasma containing the sustained pressor principle.

Grateful acknowledgement is made to Mr. R. J. Parker, Mr. W. R. Cherry and Mr. C. Wilson for their technical assistance.

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MECHANICS OF HUMAN ISOLATED VOLUNTARY MUSCLE

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Received for publication October 2, 1947

The present report describes certain experiments made upon (essentially) isolated voluntary muscles of the human subject[1]. Opportunity will also be taken to discuss certain matters of theoretical interest in muscle physiology.

MATERIALS AND METHODS

Four German amputees and two recently operated American amputees were available for the present study. These subjects, severally, had had cineplastic muscle tunnels placed through the flexors and extensors of the forearm, the biceps brachii, the triceps and a portion of the pectoralis major. Since these muscles had been freed from their bony insertions and deprived of their compensating skeletal lever arms, they were admirably suited for the study of muscle action. By insertion of pegs through the tunnels, affixed to stirrups, tensions developed by the muscles under various conditions were directly measurable. Furthermore, the activity of the muscles was produced by voluntary effort, and thus the pattern of neurostimulation was normal.

A new type of dynamometer was constructed to measure tensions, consisting of calibrated aluminum rings to which were affixed SR-4 type A-1 electrical strain gauges. The distortion of the rings was less than 0.001 inch for a force of 10 pounds. The gauges were connected in a conventional Wheatstone bridge arrangement so that all gauges were additive and the bridge self-contained on the ring. This type of connection reduced to a minimum the effects of temperature drift and external connection variations. The galvanometers used for recording bridge unbalances were Heiland type A, and the recording instrument was the Heiland type SE-301 R-12 oscillograph.

To control the possibility that the subject on approaching his maximal effort might unconsciously draw the extremity away from the dynamometer, an additional ring (compression ring) with affixed strain gauges was so placed as to record the reacting compression force from the distal end of the stump. Should no compression force be registered, it indicated that the subject was pulling away from the apparatus and that spuriously high tensions were being registered.

The experimental studies were divided into two groups: isometric muscle tension and isotonic (load-excursion) contractions.

[1] These experiments constitute a portion of the Prosthetic Devices Research Project, College of Engineering, University of California, Berkeley, under contract VAm-21223, National Research Council, Committee on Artificial Limbs. The project was under the general direction of H. D. Eberhart, Associate Professor of Civil Engineering. Thanks are due to the many workers who participated in the research.

In the isometric studies the subject was placed in such a position that the dynamometer was in line with the direction of contraction of the muscle being studied. The adjacent bony parts were placed against the dynamometer so that compression could be recorded, and the peg through the muscle was con-

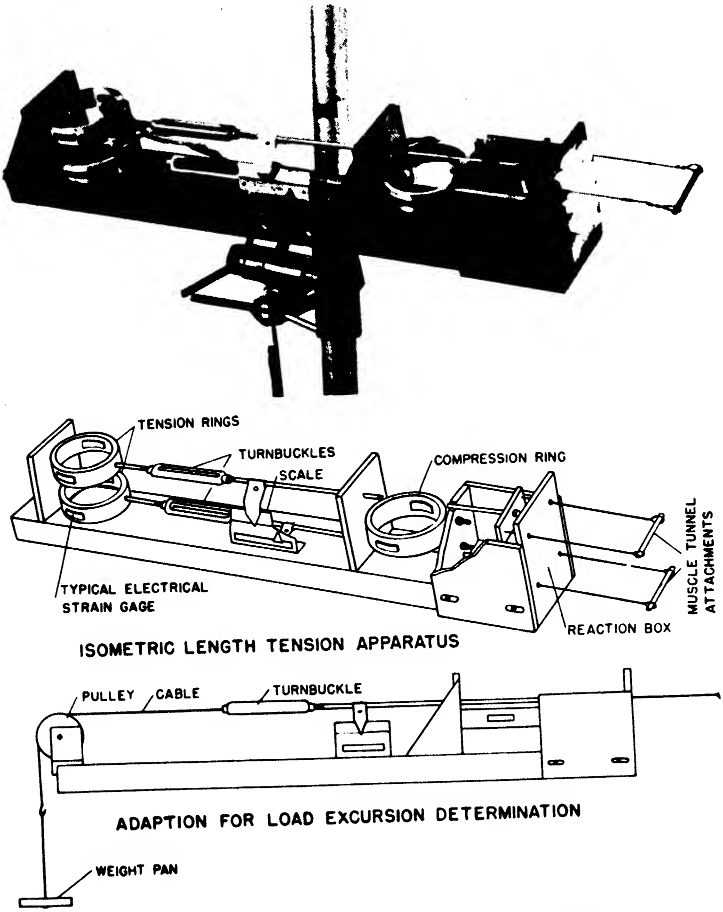


FIG. 1. STRAIN GAUGE DYNAMOMETER. *Top and middle:* arrangement for isometric studies. *Bottom:* adaptation for load-excursion studies.

nected by the stirrup and bicycle spokes to the aluminum rings. A turnbuckle and scale were inserted in the system in such a manner that passive changes in length of the muscle could be produced and measured. Recordings from the strain gauges could now be taken under conditions of passive stretch and under maximal voluntary contraction. Electromyograms, using surface electrodes, were routinely recorded. A rise of the integrated action potential to the same

maximum for successive runs assured equality of muscular effort. It was found that there was good agreement (within about 10%) between the tension developed at a given length of muscle and the integrated action potential. Electrical activity of muscle was never observed during passive stretch.

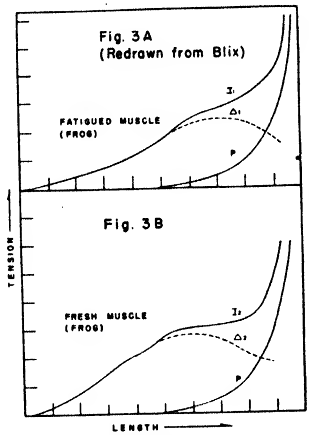
FIG. 2. DYNAMOMETER IN USE. *Above:* biceps brachii. *Below:* pectoralis major.

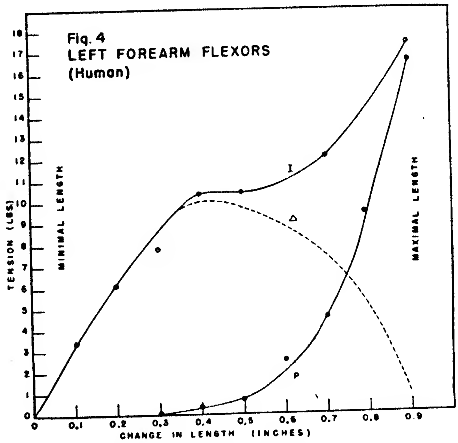
Load-excursion experiments were carried out on the same instrument, except that the tension rings were removed and replaced by a pulley and light cable to which was attached a weight pan. Increasing weights were added to the pan to produce passive stretch. The subject rather slowly (2–5 seconds) lifted each weight as far as possible and tried to maintain a uniform speed of contraction.

Details of the dynamometer and the experimental setup are shown in figures 1 and 2.

RESULTS AND DISCUSSION

1. *The isometric length-tension diagram.* Figure 4 shows the relationship between length and tension in the passively stretched (curve *P*) and the isometrically contracted (curve *I*) flexors of the forearm. Since the absolute lengths of the muscles were not known in the present experiments, the changes in length have been plotted, beginning with minimal length in the resting and contracted states. A third curve has been added (curve Δ), which is obtained by subtracting the passive tension from the isometric tension at corresponding lengths. This curve has been variously called the 'contractile force', the 'developed tension' and the 'extra tension' curve by previous investigators, and has been extensively discussed (1–7).





FIGS. 3A, 3B. PASSIVE (P) AND ACTIVE (I) LENGTH-TENSION CURVES obtained from frog muscle by loading the muscle, then tetanizing and unloading. 3A, fatigued. 3B, fresh. See discussion in text.

FIG. 4. ISOMETRIC (I), PASSIVE (P) AND DEVELOPED TENSION (△) CURVES for the flexor muscles of the human forearm.

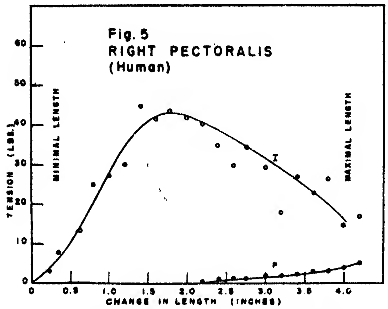
Blix (1), in his studies of frog muscle, pointed out that if the purely passive tension of a stretched resting muscle were independent of the additional force developed upon contraction (for which view he believed there was considerable justification), it followed that subtracting the passive curve from the isometric curve would yield a curve representing the contractile force. Pursuing this matter further, he showed that the Δ-curve exhibited a maximal value in the neighborhood of the 'natural' or resting length of the muscle, this being the length at which tension in the resting muscle first began to appear as the muscle was stretched. In figure 4 the resting length would correspond to the abscissal value 0.3.

It is evident that the Δ-curve of figure 4 reproduces the characteristics of the contractile force curve as described by Blix for frog muscle. Ramsey and Street (5) show the same type of curve for the isolated voluntary muscle fiber.

Ramsey (6) has recently tried to fit the Δ-curve with a parabola. It is important to note, as Blix pointed out, that whereas the right-hand side of the Δ-curve is obtained by subtracting two experimentally definable curves, the left-hand side, as customarily drawn, assumes that the passive tension is zero. Since, however, we know nothing of the passive tension below the resting length, the form of the left-hand side of the Δ-curve is open to question.

It is of great physiological significance that the 'prosthetic length' in our subjects (that is, the length at which they liked to have their muscles placed in the artificial limb, and at which they felt they could exert the most force) corresponded closely to the resting length.

Figure 5 shows the isometric length-tension diagram for the pectoralis major. At first sight, the form of the I-curve seems very different from that in figure 4.



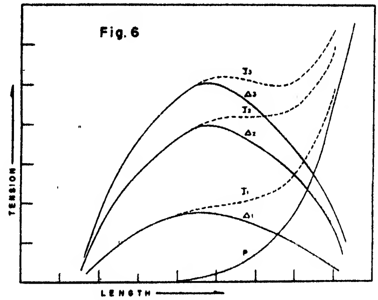


FIG. 5. ISOMETRIC AND PASSIVE TENSION CURVES for the right pectoralis major of the human subject. The developed tension curve has not been drawn, since it is practically identical with the isometric curve.

FIG. 6. DERIVATION OF A SERIES OF ISOMETRIC CURVES of various forms by adding a typical passive tension curve to each of a series of possible developed tension curves. Thus I is obtained by adding P to Δ_1. See discussion in text.

Had the experiment included measurements at much greater lengths, however, the I-curve would have started to rise again, in company with the P-curve. The pectoralis is remarkably extensible, in this experiment an elongation of over 4 inches being produced before the subject complained of discomfort. Within the range of lengths studied, the P-curve is so low that the I-curve and the Δ-curve (not drawn) are practically identical. This constitutes an experimental justification for the view that the Δ-curve is independent of the P-curve. As in figure 4, the maximum of the Δ-curve occurs near the resting length.

Blix was much impressed by the variations in form which the I-curve of a given muscle exhibited in different physiological states (freshness, fatigue, etc.) and with different experimental techniques. The results shown in figures 3A and 3B, from Blix (we have added the Δ-curves), were obtained by gradually loading a resting muscle, then tetanizing the muscle maximally and unloading it rapidly, but allowing time for the muscle to arrive at an equilibrium length at each successive load. The differences in the two I-curves Blix attributed, at least in

part, to fatigue. It will be observed that the I-curve of figure 3B shows a plateau like that of figure 4, but that the plateau is missing from the I-curve of figure 3A.

Blix pointed out that when the I-curve is obtained by the use of an isometric lever, there frequently occurs, especially if the muscle is fresh and vigorous, a secondary maximum and minimum in the curve. Such a curve is shown in figure 6, I_3, and in figure 7, the latter having been plotted from data given by Blix.

We explain[2] these variations in form of the I-curve as follows: referring to figure 6, draw a typical passive stretch curve, P. Since all studies agree that the Δ-curve, representing developed tension, is convex upward, with a maximum near the resting length, we may draw a series of possible Δ-curves, each having

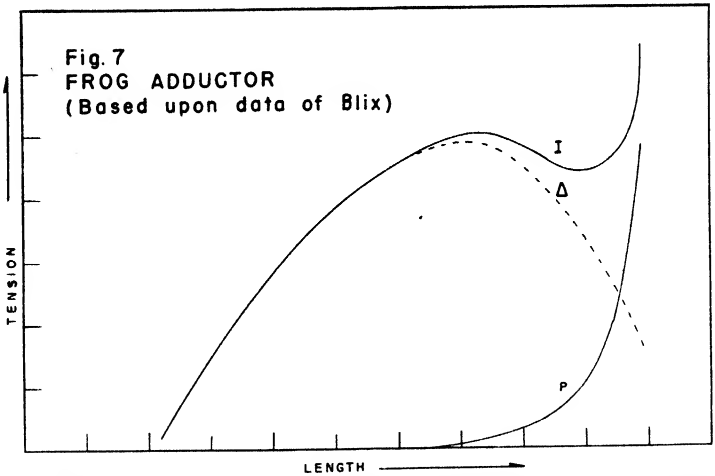


FIG. 7. ISOMETRIC LENGTH-TENSION DIAGRAM for frog muscle, showing a marked secondary maximum and minimum in the I-curve. Compare with I_3 of figure 6.

the proper general form, but differing in maximal height (Δ_1, Δ_2, Δ_3). The lowest Δ-curve would correspond to a weak or fatigued muscle, the highest to a fresh or vigorous muscle. We may then construct a series of I-curves by adding the tensions of the P-curve to those of the respective Δ-curves. The resulting I-curves differ markedly in form, these various forms being similar to those observed experimentally (figs. 4, 5, 7). The particular form of the I-curve is, however, of no special physiological significance, aside from indicating the ability of the muscle to develop tension in excess of the passive tension.

Blix pointed out that the experiment of figures 3A–B (see description above) is incapable of revealing the presence of a secondary maximum and minimum,

[2] During the course of preparation of this paper, we discovered that Buchthal (7) had given a similar explanation. Since his discussion was very brief, and not clearly implemented, we have felt justified in retaining the discussion in the present paper.

since each successive point on the I-curve represents a smaller load. This is an elegant example of the subtle manner in which a particular experimental technique may alter the nature of the experimental results.

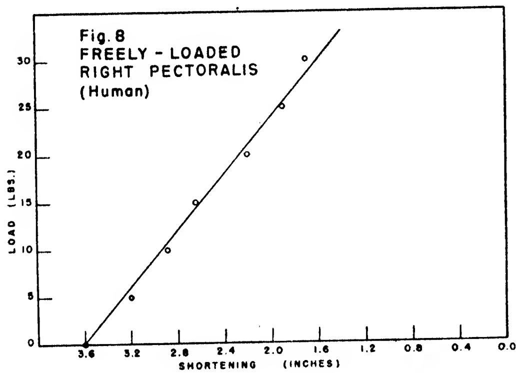
I-curves like those of figures 4, 5, 6 and 7 are of great physiological interest. The presence of a plateau or of a secondary maximum and minimum is reminiscent of the 'yield points' described for certain engineering materials. Buchthal *et al.* (8) have suggested that 'yielding', in physiological muscular activity, ensures constant contraction tension over a large range of stretch. We are of the opinion, however, that this probably is not of as much importance in the intact body as the nice compensation which occurs between the mechanical advantage of joints in various positions and the forces exhibited by the muscles at those positions. Thus, when the arm is extended, the mechanical advantage for flexion is low, but the total force (contractile plus passive) which may be exhibited by the flexor muscles is high. The reverse is true when the arm is moderately flexed. In this connection, we may remark that the view sometimes expressed in the literature (for example Fenn, 9) that the resting length of muscles in the body is practically the same as the maximal extended length, is certainly not correct. Thus the resting length of the flexor muscles of the forearm corresponds to an angle at the elbow of approximately 90 degrees (10).

Buchthal and Kaiser (11) have found that the 'dynamic stiffness' of both the resting and the contracted isolated muscle fiber is a linear function of tension. Stiffness they define as $\frac{\Delta T}{\Delta L}$, where T is tension and L is length. $\frac{\Delta T}{\Delta L}$ is therefore the slope of the tension-length curve. In their experiments they cause a muscle fiber to minutely shorten and lengthen at a rapid rate (100 cycles/sec.) and obtain therefrom a measure of the vibrational elasticity of the fiber, analogous to the sonic elasticity of engineering materials. Their result, put in the notation of infinitesimals, is that $\frac{d^2T}{dTdL}$ = constant. On integration, it follows that L is a linear function of log T (i.e., T is an exponential function of L). We have plotted L against log T for one of our smoothest and most complete passive stretch curves (P of fig. 4) and found that the points fall along a straight line. This particular 'static' experiment, therefore, agrees with the 'dynamic' findings.

Schoepfle and Gilson (12) have recently described an experiment on the retractor penis muscle of the turtle, suggesting that the elasticity of active muscle is the same, or nearly the same, as that of resting muscle at the same tension, and have thereby been led to develop a transverse expansion theory of muscle contraction. We do not wish to dispute this theory, since there may be a good deal to be said in its favor. However, we do wish to call attention to the great importance of examining in detail any experiment dealing with the elastic characteristics of muscle. The study of muscle elasticity has been intensively pursued ever since the time of E. Weber, a century ago, and anyone who reads the literature on the subject cannot fail to be impressed by the vast inconsistencies and contradictions in the results of the various investigators. Fenn (9), one of the leading students of muscle physiology, has ascribed these discordant findings

to difficulties of interpretation. We should like to amplify this comment by saying that the particular muscle used, the conditions under which it is studied and the particular experimental technique employed are of absolutely critical importance in determining the results obtained. Blix was acutely aware of this.

Referring to figure 6, it is clear that if we are working with a muscle at a length well above the resting length, we may expect to find closer correspondence between the elastic characteristics of the resting and contracted muscle than at shorter lengths, since the I- and P-curves approach each other at greater lengths. More than that, when the Δ-curve is low, the I- and P-curves approach each other very rapidly. The data in the paper of Schoepfle and Gilson suggest that they were working in a region of the I- and P-curves where the slopes of the curves were nearly the same. Furthermore, it appears likely that quick-



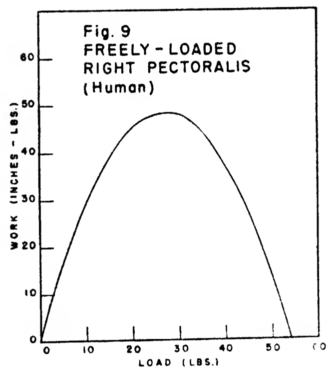


FIG. 8. LINEAR RELATION BETWEEN LOAD AND EXCURSION of the freely weighted right pectoralis major of the human subject.

FIG. 9. PARABOLIC RELATION BETWEEN LOAD AND WORK for the muscle of figure 8. The values used in calculating this curve were determined from the straight line of figure 8, extrapolated out to zero shortening.

release experiments of the type under consideration may be self-defeating, because of the momentary disappearance of contractile force during the release.

2. *The isotonic (load-excursion) diagram.* Figure 8 shows the relation between load and shortening in the freely weighted pectoralis major. In constructing this diagram, the usual conventions of graphical representations have been violated in order to represent load and length in a manner similar to that of the preceding diagrams. It will be seen that under the conditions of the experiment, and with the range of loads used, the shortening bears a linear relationship to load. The straight line, when extrapolated out to zero shortening, yields a value for the load which agrees, within experimental error, with the maximal isometric tension as previously determined. The linear relation between load and excursion is true of the other muscles studied.

3. *The load-work diagram.* Figure 9 shows the load-work diagram for the muscle of figure 8. The curve is a parabola. In calculating the curve, values

of load and shortening were read off the straight line of figure 8, extrapolated out to zero shortening. It can easily be proved that a parabola will result, as follows: since load (l) is a linear function of shortening (s), $l = ms + b$, the equation of a straight line, where m is the slope and b the l-intercept. Hence work = load × shortening = $s(ms + b) = ms^2 + bs$, which is the equation of a parabola. Since l is a linear function of s, plotting $ms^2 + bs$ against l must also yield a parabola.

SUMMARY

1. A new type of isometric dynamometer is described, in which muscle tensions are measured by strain gauges affixed to heavy metal rings and recorded oscillographically.

2. Isometric length-tension diagrams, load-excursion curves and load-work curves have been obtained for certain (essentially) isolated human muscles containing cineplastic muscle tunnels, under conditions of voluntary contraction.

3. It is shown that the isometric length-tension diagram and the load-work curve are similar to those previously described for frog muscle.

4. It is shown that the curve of developed tension has a maximum in the neighborhood of the resting length of the muscle, in agreement with earlier studies on frog whole muscle and single fibers. Attention is called to the lack of precise information concerning the form of the curve of developed tension below the resting length.

5. Variations in form of the isometric tension curve are discussed, and shown to be due to the variation in height of the curve of developed tension.

6. Evidence is provided that the curve of passive stretch represents an exponential function.

7. It is suggested that in studies of muscle elasticity, close attention should be paid to the region of the tension curves in which the muscle is being studied.

8. Under conditions of relatively slow and uniform speed of contraction, the excursion of the freely weighted muscle is shown to be a linear function of load, and proof is offered that a parabolic relation between load and work necessarily follows from this relationship.

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OBSERVATIONS ON THE EFFECTS OF ADRENALIN ON RENAL FUNCTION AND CIRCULATION IN MAN

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Received for publication August 28, 1947

It was shown in 1922 (8) that adrenalin caused a swelling of the perfused rabbit's kidney and an increase in perfusion pressure when blood was supplied at a constant rate of flow, an observation that was confirmed on the eviscerate rabbit and dog (9). Richards and Plant explained this apparent paradox by postulating that adrenalin acts preferentially on the efferent glomerular vessel. Observations on the heart-lung-kidney preparation (13) have confirmed this view. Chasis and his co-workers (4) have shown that in man the action of adrenalin is to cause a constriction of the efferent glomerular vessel. The relation of these findings to the normal haemadynamics of the kidney has been discussed by Smith (10).

During exercise there is a marked diminution in renal plasma flow and glomerular filtration rate (2) and in an attempt to elucidate the possible rôle of adrenalin in producing such changes the effects of the administration of the hormone on the renal haemadynamics in man have been re-investigated.

METHODS

The subjects were students with normal renal function. Experiments were carried out during the afternoon, following a light lunch. Eight hundred milliliters of water were given by mouth to ensure adequate urine flow. Renal plasma flow and glomerular filtration rate were determined by a standard technique (3). After two or more control periods, adrenalin was administered to six subjects intramuscularly (0.75–1 mgm.) and to eight subjects by a continuous intravenous infusion (0.0005–0.01 mgm/min. for periods varying from 25–40 minutes). Blood pressure was determined by auscultation and pulse rates were taken at intervals.

RESULTS

Renal haemadynamic changes. The changes in renal plasma flow, glomerular filtration rate, and filtration fraction for the group receiving adrenalin intramuscularly are given in the table. There is a fall in renal plasma flow accompanied by a consistent rise in filtration fraction. Since the time in which the adrenalin was absorbed into the circulation following intramuscular injection cannot be determined exactly, adequate control and interpretation of the experiments was difficult. In subsequent experiments, therefore, adrenalin was given intravenously. The changes observed in this group of subjects will also be seen in the table. Again the most marked changes are the fall in renal plasma flow

and the rise in filtration fraction. Following both intramuscular and intravenous adrenalin, a fall in the average glomerular filtration rate was observed but was within the normal limits of variation.

In figure 1 a similarity between the effects of adrenalin and pain (14) on renal dynamics will be noted and also between those in recovery from exercise (2) and *grade 2* hypertension.

TABLE 1

SUBJECT	SEX	SURFACE AREA	DOSAGE	GLOM. FIL. RATE CC./MIN.			RENAL PLASMA FLOW CC./MIN.			FIL. FRACTION %		
				Control	After adrenalin	% Change	Control	After adrenalin	% Change	Control	After adrenalin	% Change
Subjects receiving intramuscular adrenalin												
			mgm.									
1	M	1.80	0.5	161	103	−36	700	298	−57.5	23	34.6	+50.5
2	M	1.77	0.5	136	139	+2.2	922	891	−4.22	14.7	15.6	+6.13
3	M	1.95	0.5	157	70	−55.4	862	258	−70	18.2	27.1	+49
4	M	1.80	0.75	107	114	+6.5	777	381	−51	13.8	30	+117.4
Average				155[1]	106	−24.3	734[1]	457	−44	16.9	26.8	+58.5
Range				107–161	70–139	—	700–922	258–891	—	13.8–23	15.6–34.6	—
5	M	1.7	0.5	—	163	—	—	756	—	—	21.6	—
6	M	1.9	0.8	—	223	—	—	644	—	—	34.6	—
Subjects receiving intravenous adrenalin												
7	M	1.64	0.0025	185	136	−26.4	512	204	−60.2	36	66.8	+85.6
8	M	1.83	0.0025	231	194	−16	668	445	−33	34.6	43.5	+26.3
9	F	1.67	0.004	90	118	+31.2	516	430	−16.7	17.4	27.4	+57.5
10	M	1.63	0.005	200	130	−35	852	243	−71.5	23.5	53.5	+127.7
11	M	1.85	0.0005	116	102	−12.1	667	426	−36.2	17.4	24	+38
12	M	1.83	0.005	136	89	−34.6	861	653	−24.2	15.8	13.6	−13.9
13	M	1.9	0.005	203	187	−12.5	600	291	−51.5	33.8	64.5	+91
14	M	1.9	0.01	144	148	+2.8	860	449	−48	16.7	33	+98
Average				155[1]	138	−15.3	734[1]	392	−43.5	24.4	40.8	+67.3
Range				90–231	89–194	—	512–861	204–653	—	15.8–36	13.6–66.8	—

[1] Average for whole series.

Effect on blood pressure, pulse and peripheral vascular system. A rise in pulse pressure was noted in every case and, in all but one, this rise was followed by an increase of pulse rate averaging 20 beats per minute, the greatest being from 50 to 90. Three subjects receiving 0.005–0.1 mgm/min. intravenously complained of palpitations, even though no extra systoles or increase of pulse rate were noted. Most subjects showed a cutaneous vasoconstriction; in six, the vasoconstriction was followed by generalized cutaneous hyperaemia. Five subjects developed a coarse generalized tremor following the intramuscular injection and three a similar type of tremor during the intravenous infusion.

Phosphate, chloride and water reabsorption and excretion. Adrenalin caused a rise in the plasma level of inorganic phosphorus in 50% of the cases, but in the

remainder the plasma level remained constant or fell. There was no relationship apparent between the amount of adrenalin and the effect on plasma phosphate. No effect, moreover, was noted on tubular activity towards phosphate nor towards chloride, other than those which are observed in a simple diuresis (2).

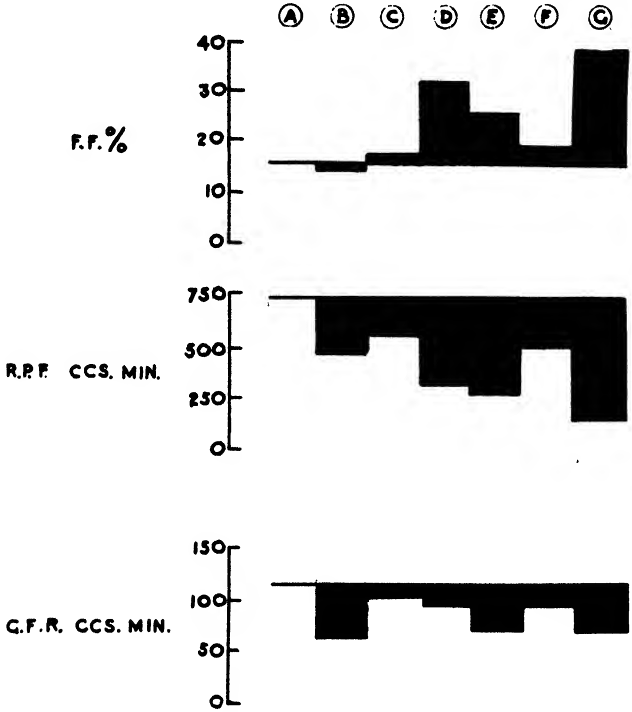

FIG. 1. SCHEMATIC REPRESENTATION OF RENAL DYNAMICS in certain physiological and pathological states. *A*. Control observations (2); *B*. During exercise (2); *C*. During the recovery from exercise (2); *D*. During the administration of adrenalin; *E*. Grade 4 hypertensives, unpublished observations; *F*. Grade 2 hypertensives, unpublished observations; *G*. Pain (14).

Following intramuscular adrenalin at the onset of the experiment in two subjects, there was no change in the subsequent urine flow, for the diuresis, both in duration and amount, was comparable to those noted in our previous control series. On the other hand, in four subjects there was a marked drop in urine flow associated with changes in clearance which were certainly due to adrenalin. In the remaining subjects, no significant change was apparent.

DISCUSSION

The fraction of the total plasma flow through the kidney, which is filtered at the glomerulus, is primarily dependent upon the mean intraglomerular pressure,

which may be varied by changes in the tone of the afferent and efferent arterioles. If the afferent be constricted, blood flow is reduced with consequent fall of the intraglomerular pressure. If, however, the efferent be constricted, the intraglomerular pressure rises, even though the total kidney flow is reduced. A diminished filtration fraction will, therefore, result from a fall in intraglomerular pressure and an increase will follow a rise. Hence the finding of decreased blood flow and an increased filtration fraction, following the administration of adrenalin, should imply constriction of the *efferent* glomerular vessel. We have shown elsewhere that a fall in renal plasma flow and filtration fraction occurs during exercise, suggesting an increased tone in the *afferent* glomerular vessel. In both of these two series of experiments, the changes in filtration fraction were so consistent as to rule out a purely fortuitous finding. Therefore, it appears unlikely that adrenalin could be primarily responsible for the changes occurring in exercise. It is true that in the recovery phase after exercise, filtration fraction increases while glomerular filtration rate and renal plasma flow remain depressed. Only in this phase does there appear any similarity between the effect of exercise and that of adrenalin.

The rise in blood pressure and pulse rate following adrenalin, in our experiments, was not comparable in any degree to those seen in exercise and, since larger doses would have produced more marked tremor and weakness, it seems improbable that adrenalin can play any major part. This does not deny that marked sympathetic activity or the release of adrenalin may occur in exercise, but makes it improbable that the physiological amounts of adrenalin produced in such circumstances can cause the circulatory and renal changes noted.

It is possible that the parenteral injection of adrenalin does not exactly mimic the effects of sympathetic stimulation and that some more potent factor masks the adrenalin effect. Similar effects, however, to that of parenteral adrenalin can be reproduced by the body itself in two ways: *a*) repeated painful stimuli (14) may cause a fall in plasma flow and rise in filtration, and *b*) changes may occur in apprehensive subjects in every way similar to that of intravenous adrenalin. Smith (11) has also drawn attention to the effects of apprehension, a possibility which can never be adequately excluded in untrained subjects. Such reactions have been attributed to the extra production of adrenalin and may serve to establish the similarity in action of parenteral adrenalin and adrenalin spontaneously produced.

It has frequently been stated that adrenalin causes an inhibition of urine flow (7, 12). From our experiments we have been unable to reach any definite conclusion, for it is often difficult to decide whether urine flow has become reduced as a result of adrenalin or as a result of the natural termination of diuresis. For similar reasons, the data of other workers (5, 11) fail to give any clear indication. Further inconclusive evidence was provided in class experiments when injections of 0.5–1 mgm. of adrenalin intramuscularly produced a definite inhibition of urine flow in only 3 out of 25 subjects. It would appear, then, that adrenalin in the doses we have used has no dramatic effect on urine flow in man. The exact effect produced can only be decided by conducting an experiment in such a way that a

maintained diuresis is set up. If parenteral adrenalin then causes a diminution of urine flow with a subsequent recovery, an inhibitory effect would be shown.

Our observations as to the effect on urine flow would appear to be at variance with those made on dogs (7). Other observations on the effects on the general and renal circulation in animals (7, 12) are also at variance with our findings and with those of McMichael and Sharpey-Schafer (6) and Allen, Barcroft and Edholm (1). The discrepancy may be either due to the higher dosage and faster administration in experimental animals or to a fundamental difference in the reaction to adrenalin in man and lower animals.

SUMMARY

The effects of intravenous and intramuscular adrenalin have been studied in man, and results confirming those of Chasis *et al.* have been obtained. A fall in renal plasma and increase in the filtration fraction were constant effects, while inhibition of urine flow was inconstant. There was no evidence that adrenalin plays a significant part in the renal picture seen in exercise.

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THERMAL EXCHANGES OF MAN AT HIGH TEMPERATURES

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Received for publication August 9, 1947

Studies of the physiological responses of men to high environmental temperatures require for their most general application a means of transfer of data secured under particular environmental conditions to other intermediate but untested conditions. This need would be fulfilled if there were available functional relationships capable of describing thermal stress to the man in terms of the various environmental factors. Such relationships for limited ranges of environmental conditions are available for convection (1a, b, c, d) and for still more limited ranges for evaporation (1d, e). Thermal exchange by low temperature radiation appears to be well founded on both theoretical and experimental grounds (1f, 2a). The need for descriptive relationships of thermal exchange has led to attempts to extrapolate the meager data now available to conditions out of the range of the original experiments (3).

The ideal procedure for establishing these relationships is by means of complete calorimetry. The technical difficulties and elaborate equipment involved in this approach become almost prohibitive when higher wind velocities and working subjects are studied. The simpler method of partial calorimetry has been used at the Pierce Laboratory with considerable success over normal temperature ranges (1). This approach is less satisfactory at more severe environmental conditions largely because of the greater difficulty of reaching thermal equilibrium and the consequent higher rates of storage (subject to considerable error in estimation). However, the potential usefulness and need (especially during the war) of even roughly quantitative descriptions of convection and evaporation at high thermal loads justifies their study by the method of partial calorimetry. The results of such a study are presented in this report.

The principle involved in the use of partial calorimetry to allocate thermal exchange into its several components is contained in the statement that at equilibrium (no increase or decrease in heat content of the body) the rate of thermal flow outward across the envelope of reference (skin) is equal to the rate of thermal flow inward, or, in the absence of thermal equilibrium, that these two rates differ by the rate of change in the heat content of the body. These considerations apply regardless of the source of heat (metabolism, environment, stored heat) or the nature of its transfer (convection, conduction, evaporation).

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This statement can be mathematically expressed as $M + S + E + C + R = 0$ where the symbols represent, respectively: M, the rate of metabolic heat production, always positive in sign; S, the rate of storage (the rate of gain or loss in heat content of the body), positive in sign when the body heat content decreases, negative when heat is gained by the body; E, the rate of evaporative heat loss, always negative in sign; and C, the rate of thermal exchange by convection, and R by radiation, both positive when delivering heat to the body and negative when removing it. Of these variables, M can be determined from the rate of oxygen consumption; S from the changes in rectal and skin temperature, unfortunately, with uncertain reliability; and E from the evaporative weight loss of the subject and the latent heat of vaporization. With M, S, and E available, the sum of $C + R$ can be calculated by difference. The separation of C from R can be accomplished mathematically by taking advantage of the fact that R is independent of wind velocity, or alternatively R can be calculated from skin and wall temperatures by accepted principles and subtracted from the sum $C + R$.

Utilizing the above principles, 4 subjects were studied while standing nude, standing clothed and walking clothed at 5 wind velocities in each of 7 environmental conditions, representing 3 moisture contents at 5 air temperatures (table 1). It has been possible to make a fairly complete analysis of the standing-nude experiments. The data from the clothed experiments give less satisfactory results.

Test conditions and procedures. The experiments were carried out on 4 healthy young men whose physical characteristics are given in table 2. After preliminary training in the cool, they were trained and acclimatized to heat by working for 4 hours per day as follows: 4 days at D.B.[5] 120°F., W.B.[6] 78°F.; 2 days at D.B. 120°F., W.B. 86°F.; 2 days at D.B. 94°F., W.B. 91°F.; 1 day at D.B. 96°F., W.B. 92°F.; and finally 1 day at D.B. 120°F., W.B. 88°F. During this period test clothing was worn and activity and environment were at least as severe as during the actual test days. Acclimatization produces well adjusted subjects with minimal changes in heat storage.

Three subjects were used, with the fourth held in reserve, but helping in the hot room and receiving the same exposure as the 3 test men. On the eleventh test day the man in reserve replaced an original subject who was removed because of an upper respiratory infection. With this exception, the subjects were in good condition throughout the study. They spent 7½ hours in the hot room each test day, but slept in barracks maintained at normal temperatures. Data were collected on 5 days in each week. Sunday was spent out of the hot room and Monday was devoted to reacclimatizing 4-hour marches.

The 7 environments were studied in a regular sequence, one wind velocity being covered each day (table 1). The completion of each cycle of 7 environments was followed by a 'Base Day'. The coefficients of convection, radiation and evaporation for all 'Base Days' agreed well with each other, indicating that the

[5] D.B. dry bulb temperature.

[6] W.B. wet bulb temperature.

TABLE 1. *Environmental conditions studied*

Number in parentheses, environment code number; number with degree sign, wet bulb temperature F.

DRY BULB TEMP.	VAPOR PRESSURE mm.Hg		
	13	25	36
°F.			
90	(1) 69.5°	X	X
96	(2) 71.5°	(5) 82.8°	(7) 91.0°
105	(3) 74.0°	X	X
120	(4) 78.0°	(6) 88.0°	X

Wind velocities

CODE	FT/MIN.
a	30
b	75
c	150
d	300
e	600

Sequence of environments

The number refers to environment, the letter to wind velocity.

WEEK[1]	M(ACC)[2]	T	W	T	F	S
1	120–88	Bd[3]	6a	7e	5e	2a
2	120–78	1c	3e	4a	Bd	6c
3	96–91	7e	5a	2c	1e	3a
4	120–78	4c	Bd	6e	7a	5c
5	120–78	2e	1a	3c	4e	Bd
6	120–88	6b	7d	5b	2d	1b
7	120–88	4d	Bd	6d	7b	5d
8	120–88	2b	1d	3d	4b	Bd

[1] Starting 9 October, 1944.
[2] Acc = Re-acclimatization, no data collection.
[3] Bd = Base Day; D.B. 120°F—W.B. 88°F. 300 ft/min.

TABLE 2. *Physical characteristics of the subjects*

SUBJECTS	COMPLEXION	AGE	HEIGHT	WEIGHT	SURFACE AREA
		years	*cm.*	*kg.*	M^2
Mil	light	20	166	62.4	1.68
Lon	light	22	170	65.5	1.75
God[1]	brunette	20	185	62.5	1.82
McG	brunette	22	177	74.4	1.90

[1] God replaced McG after 11 test days.

physiological response of the subjects to the same conditions remained reasonably constant throughout the study. During the 8 weeks working metabolism fell by 10%; rectal temperatures and heart rates showed little consistent change.

The tests were carried out in a sheet-metal wind tunnel ($5\frac{1}{2}$ ft. wide, $7\frac{1}{2}$ ft. high and 20 ft. long) in the hot room. Six 24-inch fans at the discharge end of the tunnel produced air flow, the velocity of which was changed by adjusting either the fan speed or the louvres (located just upstream from the fans) or both. The entering end of the tunnel was packed over the entire section with 30-inch lengths of 8-inch galvanized pipe lying in the axis of the tunnel. This served as an air straightener and protected the inside of the tunnel from outside air disturbances. Air movement was virtually uniform across the cross-section of the tunnel to within 6 inches of the walls. A treadmill on which the subject stood or walked constituted the central portion of the tunnel floor. The inside surfaces of the tunnel were painted flat black. Dry and wet bulb temperatures inside the tunnel were maintained at the designed conditions plus or minus 1°F. and were uniform laterally. Vertically there were gradients between head and floor levels of 1°F. or less for the cooler situations and not more than 3°F. for the hottest conditions.

On test days 3 separate experiments were performed on each of the 3 subjects in the same sequence: walking clothed in the morning, and standing nude and standing clothed in the afternoon. The subject always faced into the air flow and was accompanied in the tunnel by one observer who remained behind the subject at all times.

The walking tests were performed on the treadmill at 3 m.p.h. and a 3% grade. This led to metabolic rates of approximately 160 Cal/M^2/hr. The standing metabolic rates were in the range 40–60 Cal/M^2/hr.

All test periods were 30 minutes long and were preceded by an equilibrating period designed to reduce storage during the test period. Before the walking experiments the equilibrating period consisted of a 60-minute walk on the hot room track (2.7 m.p.h. carrying a 20-lb. pack) followed by a 10-minute walk on the treadmill at the test wind velocity. Before the standing experiments it consisted of a 10-minute stand outside the tunnel either clothed or nude.

During the clothed tests the subjects wore well laundered, two-piece, herringbone twill (HBT) fatigue uniforms, light wool socks, cotton underwear shorts and field shoes. To avoid sweat loss by drippage the jacket was tucked into the trousers, the trouser legs into the sock tops, and the jacket cuffs into 4-inch wristlets made of sock tops. In the nude experiments the subjects stood on wooden clogs in a shallow tray containing mineral oil which collected the dripping sweat. In the clothed experiments a dry suit was donned immediately at the start of the test period just after the equilibrium period. In each experiment water salted to 0.1% was given in amounts approximating sweat loss.

Data collected. The environmental conditions inside the wind tunnel were determined during each test period as follows: *a*) wet and dry bulb temperature, 6 feet and 1 foot above floor level, three times per test period, by calibrated motor-driven psychrometers; *b*) wall temperature, of the 6-tunnel surfaces, by radiometer at the beginning and end of both the morning and afternoon tests; *c*) velocity of air flow at a point waist high, 4 feet in front of the subject, twice each period by a velometer and 3 times each period by hot wire anemometer.

The following data were obtained on each subject: *a*) rectal temperature by

calibrated clinical thermometers at the start and end of each test period; *b*) skin temperature at the start, mid-point and end of each test period by radiometer when nude, by contact thermocouples when clothed; *c*) clothing temperature by radiometer at the same time as skin temperature; *d*) oxygen consumption in the walking tests during the first and last 10 minutes of each period by an open circuit system, and in the standing tests for the entire 30 minutes by a closed circuit system; *e*) heart rate at the beginning, mid-point and end of each period by palpation; *f*) evaporated sweat loss, determined by the difference in weight at the start and end of a test period of the subject plus his accessories (clothing in the walking experiments, clothing and towel in standing clothed experiments and towel and drip pan in nude experiments); *g*) total sweat loss, the evaporated sweat loss plus the increase in weight of the accessories above mentioned.

Treatment of data. Weighted skin and surface temperatures were calculated for each of the 3 sets of readings in each period according to the weighting factors shown in table 3. These factors are based on the surface area measurements of Hardy and Dubois (2b). The necessary readjustments required by the small number of zones measured were made by grouping unmeasured zones with those measured zones which in previous studies had been observed to have similar temperatures, admittedly a dangerous expedient. It receives some justification, however, in that at the high temperatures here observed, the maximum range of variation of skin temperatures from zone to zone is small. The emissivity of both skin and clothing was taken as unity. The initial and final weighted skin temperatures were used in the calculation of storage and the average of the 3 values per period was used in calculation of vapor pressure and temperature gradients.

The 6 readings of dry bulb temperatures in each period were averaged to give the value used. The wet bulb temperature was similarly obtained. Vapor pressure was calculated from these averaged dry (T_a) and wet bulb temperatures (T_{wet}) by the formula:

$$P_{H_2O} = P_{H_2O\,T_{wet}} - 0.265\,(T_a - T_{wet}).$$

This expression was based on calibration of the psychrometers used in this study against dew-point measurements.

Wall temperature was taken as the mean of the measurements of the six surfaces, and the average of the initial and final wall temperatures thus calculated was used. Wall temperatures deviated only slightly from air temperatures.

Air velocity was obtained by averaging the 5 measurements made per period.

The heat equivalent of the oxygen consumption was calculated in the usual way; the actual R.Q. was used to determine the caloric equivalent of oxygen in the open circuit runs, while the value 4.83 Calories/liter O_2 was used in the standing experiments.

The actual interval between the initial and final weights of the subject were used in calculating evaporation and sweat rates. This interval was longer than the tunnel exposure by about 2 minutes. Sweat loss, total and evaporated, was calculated from weight differences and water intake, corrections being made for

Table 3. *Factors used for calculation of weighted skin and surface temperatures*

Hardy, DuBois Areas		Zones measured	Weighting factors				
			Nude Skin T_s	Clothed			
				Standing		Walking	
Zone	Area			Skin T_s	Surface T_e (d)	Skin T_s	Surface T_e (d)
Head	0.07	Cheek	0.07	0.07	0.05	0.14e	0.12e
Trunk	0.35	Chest Back	0.18 0.17	0.35tb	0.19 0.17	0.35tb	0.19 0.17
Arms	0.14	Upper arm	0.14	0.14t	0.15	0.14t	0.15
Hands	0.05	Palm	0.05	0.05	0.04	0.05	0.04
Thigh	0.19	Thigh	0.39a	0.19t	0.40a	0.19t	0.33f
Legs	0.13	Calf		0.20tc		0.13t	
Feet	0.07						

t—Obtained by thermocouple; all other temperatures by radiometer.
a—Feet and legs grouped with thigh.
b—Back and chest grouped.
c—Feet grouped with calf.
d—Because of increased surface area of clothed man, head and hand factors decreased, all other factors increased.
e—Foot grouped with cheek.
f—Legs grouped with thigh.

Table 4. *Relation of clothed man surface area to nude man surface area*

Subject No.	Type[1]		Height	Weight	Size		Surface area M^2					Ratio $\frac{\text{Clothed man S.A.}}{\text{Nude man S.A.}}$
	Height	Weight			Jacket	Trousers	Jacket	Trousers	Jacket & trousers	Clothed man[2]	Nude man	
			cms.	*kgm.*								
1	S	L	163.8	45.0	34R	30–33	0.875	1.057	1.932	2.208	1.45	1.52
2	I	L	168.9	57.5	34R	32–33	0.981	1.146	2.127	2.439	1.64	1.48
3	T	L	183.5	57.3	36R	32–33	1.038	1.206	2.244	2.577	1.75	1.47
4	S	I	157.5	60.5	34R	30–33	0.830	1.057	1.887	2.191	1.60	1.36
5	I	I	172.1	68.2	34R	32–33	0.930	1.167	2.097	2.437	1.79	1.36
6	T	I	193.0	89.2	38L	34–33	1.086	1.180	2.266	2.680	2.18	1.22
7	S	H	161.3	66.6	34R	34–33	0.882	0.992	1.874	2.195	1.69	1.29
8	I	H	176.5	85.5	38L	38–33	1.045	1.278	2.323	2.703	2.00	1.35
9	T	H	182.9	101.4	40R	40–33	1.169	1.424	2.593	3.013	2.21	1.36

[1] S = Short; I = Intermediate; T = Tall; H = Heavy; L = Light.
[2] = Surface area of head + hands + feet + clothing = 0.19 nude man S.A. + clothing S.A.

weight loss due to excess weight of CO_2 excreted over O_2 consumed and for loss of water from the lungs. The excess CO_2 was determined by the formula, $(CO_2 - O_2)$ grams/hour $= 118 \times O_2$ (L/min)(RQ $-$ 0.727). In the standing experiments the R.Q. was taken as 0.825.

Surface area of clothed man. The problem of the clothed surface area is a difficult one involving not only the actual area but also the effective area as determined by the folds. From measurements of exposed clothing areas carried out on 9 men representing different body builds the ratio of clothed man surface area to nude surface area was calculated. The results are shown in table 4. These are maximum values since they are made on stretched clothing. It seems not unreasonable that the effective ratio would ordinarily fall in the range 1.20 to 1.35. This series did not include the subjects used in the calorimetry studies. Because of the variation from man to man, and even from time to time, depending on how the folds fall, the coefficients of thermal exchange for the clothed men have been calculated using the nude surface area. This gives the most predictable area and permits future correction should an acceptable factor for clothed men be found. Thus the coefficients for clothed men here calculated should be higher than those for nude men by the ratio of the two surface areas (1.20 to 1.35:1).

Notation, units and calculation of thermal exchange:

$C + R$ was calculated from the basic heat equation

$$M + E + S + C + R + W = 0$$

where the terms have the following significance and origin:

1. *Evaporation*

E' = Total heat exchange by evaporation, Cal/M^2/hr.
= (kg sweat loss/hr/M^2 $-$ CO_2 excess/hr/M^2) 575.

H_e = Heat exchange by evaporation in the respiratory tract, Cal/M^2/hr.[7]

E = $E' - H_e$ = Heat exchange by evaporation from the surface of the body, Cal/M^2/hr.

[7] H_e was estimated by making the assumptions indicated below as to vapor pressure of expired air and inspired spirometer air.

Walking (open circuit system):

H_e = 0.0418 $(P_a - P_1)$ VR, where
P_a = vapor pressure of tunnel air,
P_1 = vapor pressure of expired air,
VR = ventilation rate, liter/min.

Standing (closed circuit system):

H_e = 0.0418 $(P_{sp} - P_1)$ VR, where
P_{sp} = spirometer air vapor pressure taken as 90% saturated,
P_1 = 44.6 mm Hg, except for ambient temperatures of 105°F. and above, where 49.2 mm Hg was taken,
VR = ventilation rate, liter/min.; estimated from rate of oxygen consumption by a correlation between the two used in this laboratory.

$P_{(a,s,e)}$ = Vapor pressure of water in air, on skin, clothing, mm.Hg

$E/\Delta P$ = Coefficient of evaporation, Cal/M^2/hr/mm.Hg ('$(a - e)$' or '$(a - s)$' following a coefficient signify that ΔP or ΔT has been calculated from the difference between air and surface or air and skin respectively.)

2. *Convection and radiation*

$(C + R)'$ = Total heat exchange by convection and radiation, Cal/M^2/hr, defined by $(C + R)' + M + S + E + W = 0$

H_c = Heat exchange by convection in the respiratory passages, Cal/M^2/hr.[8]

$C + R = (C + R)' - H_c$ = Heat exchange by convection and radiation from the surface of the body, Cal/M^2/hr.

$T_{(a,\,w,\,s,\,e,\,r)}$ = Temperature of air, wall, skin, clothing, rectum, °C.

$\dfrac{C + R}{\Delta T}$ = Combined coefficient of convection and radiation, Cal/M^2/hr/°C.

$C/\Delta T$ = Coefficient of convection, Cal/M^2/hr/°C.

$R/\Delta T$ = Coefficient of radiation, Cal/M^2/hr/°C.

3. *Metabolism*

M = Metabolic heat production, Cal/M^2/hr.

4. *Water ingested*

W = Heat exchange by water intake, Cal/M^2/hr. = kgm. water/hr/M^2 $\times (T_{\text{water}} - T_r)$

5. *Storage*

$$S = \text{Storage, Cal/M}^2\text{/hr.} = \frac{(0.83)\ (\text{weight in kgm})\ (0.67\ \Delta T_r + 0.33 \Delta T_s)}{(\text{Time interval, hours})\ (\text{Surface area, M}^2)}$$

where 0.83 represents the average specific heat of the body and 0.67 and 0.33 are the fractional portions of the body conforming to average temperatures of T_r and T_s, respectively (2b).

Legitimate corrections were made even though their order of magnitude was low in relation to the probable error of the measurements. Thus, the weight-

[8] H_c was estimated from the ventilation rate and an assumed temperature of expired air thus:

$$H_c = 0.0187\ (T_a - T_{\text{exp.}})\ VR, \text{ where}$$

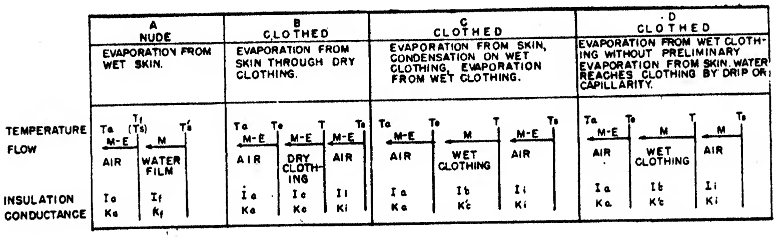
T_a = Temperature of ambient (inspired) air,

$$T_{\text{exp.}} = \frac{T_r + T_s}{2} \text{ and,}$$

VR as defined under H_e.

loss correction for excess CO_2 was at most only 12 grams/M^2/hr. H_e in the walking experiments ranged from about 5 Cal/M^2/hr. in the humid environments to about 15 Cal/M^2/hr. in the dry environments. In the standing experiments (closed circuit system) H_e was independent of ambient vapor pressure, and ranged from -1 to $+4$ Cal/M^2/hr. H_c was ordinarily less than 1 Cal/M^2/hr. in the standing experiments at 120°F, increasing in the walking experiments to about 2.5 Cal/M^2/hr.

Reliability. Granting the validity of the determination of the thermal quantities E, M and S, the question arises whether the E experimentally measured is equivalent to the E required by the basic heat equation.



	A	B	C	D
1)	$E - M = C + R$	$T_s - T = I_i(M-E)$	$T_s - T = I_i(M-E)$	$T_s - T = I_i M$
2)	$M - E = -(C+R)$	$T - T_e = I_c(M-E)$	$T - T_e = I'_c M$	$T - T_e = I'_c M$
3)	$K_a(T_a - T_s) = C + R$	$T_e - T_a = I_a(M-E)$	$T_e - T_a = I_a(M-E)$	$T_e - T_a = I_a(M-E)$
4)	$\frac{T_a - T_s}{I_a} = C + R$	$T_s - T_e = (I_i + I_c)(M-E)$	$T_s - T_e = (I_i + I'_c)\left(M - E\frac{I_i}{I_i + I'_c}\right)$	$T_s - T_e = (I_i + I'_c)M$
5)	$\frac{T_s - T_a}{I_a} = -(C+R)$	$T_s - T_a = (I_i + I_c + I_a)(M-E)$	$T_s - T_a = (I_i + I'_c + I_a)\left(M - E\frac{I_a + I_i}{I_i + I'_c + I_a}\right)$	$T_s - T_a = (I_i + I'_c + I_a)\left(M - E\frac{I_a}{I_i + I'_c + I_a}\right)$
6)	$\frac{T_s - T_a}{I_a} = M - E$	$\frac{T_s - T_e}{T_e - T_a} = \frac{I_c + I_i}{I_a}$	$\frac{T_s - T_e}{T_e - T_a} = \frac{I_i + I'_c}{I_a}\,\frac{M - E\frac{I_i}{I_i + I'_c}}{M - E}$	$\frac{T_s - T_e}{T_e - T_a} = \frac{I_i + I'_c}{I_a}\,\frac{M}{M - E}$
7)	$T_s - T_a = I_a(M-E)$			
8)	$T'_s - T_f = I_f M$			

FIG. 1. EQUATIONS OF THERMAL FLOW FOR EVAPORATION FROM VARIOUS SURFACES. In all cases it is assumed that S = 0, and that the condition C + R + M + E = 0 is fulfilled. E is always negative for the conditions considered here. The rates of heat transfer by C and by R are combined in a common coefficient K = 1/I.

Consider first evaporation from wet skin in the nude man illustrated in figure 1 A. In this situation the rate of heat flow to T_f from the environment will be equal to $K_a(T_a - T_f)$, where K_a represents the combined coefficients of C and R. Since the only other source of heat to the surface T_f is M(taking $S = 0$) and since for a steady condition of heat flow, the rate of access of heat to the surface must equal the rate of heat dissipation, the following condition is fulfilled: $K_a(T_a - T_f) + M + (-E) = 0$. Since $K_a(T_a - T_f) = C + R$, the basic equation is satisfied with respect to the surface T_f. Since radiometric measurement of the temperature of wet skin actually measures the water film temperature, the significant temperature for the surface of reference is actually obtained.

Three possible paths of evaporation from clothed men are illustrated (fig. 1 B, C, D). Equations of heat flow for these situations are developed in an analogous manner. For convenience, they are arranged as equations of temperature difference (see Burton, 6). Note that equations 3B, C and D all have the

same form $I_a(M - E) = (T_e - T_a)$. Since this is equivalent to $K_a(T_e - T_a) = M - E$, and $K_a\ (T_e - T_a) = C + R$ for the clothing surface, the required condition is fulfilled for these 3 conditions of evaporation from the clothed man, when T_e is taken as the temperature of reference for $C + R$.[9] In case a water film of appreciable thickness is present on the clothing the correct surface temperature is no longer T_e but the temperature of the water film; this is still the temperature actually measured.

The reliability of $C + R$ by thermal difference where $C + R = -(-E) - M - S - W$ is limited by the accuracy of estimation of E, M, and S. In the calculation of E, use of the same value for the latent heat of vaporization for all skin temperatures and disregard of the energy involved in vapor expansion or change in temperature lead to errors which appear to be minor relative to other uncertainties. Also relatively minor is the error involved in disregarding frictional loss in correcting M for external work in the walking experiments.

A reliable calculation of storage from the data available and by the procedure here used appears to be hopeless. The internal heat distribution undoubtedly varied during the test period, making untenable the use of any fixed distribution ratio for calculation of storage. Moreover, the assumption that weighted rectal

[9] This conclusion is not invalidated by the fact that the amount of evaporation required for steady state conditions varies with the path of evaporation and with the insulation of the various layers through which the heat must flow. The difference in evaporation can be thought of as producing different temperatures of the outermost surfaces. Thus, in the case of evaporation from wet unclothed skin (fig. 1A) the equations of heat flow through the water film and from the water surface to the environment are:

$$T_s' - T_f = I_f M \qquad \text{(skin to water film)}$$

$$T_f - T_a = I_a(M - E) \qquad \text{(water to air)}$$

which upon adding gives

$$T_s' - T_a = (I_a + I_f)\left(M - E\,\frac{I_a}{I_f + I_a}\right) \qquad \text{(skin to air)}$$

These equations show, first, that T_f is lower than the true skin T_s' temperature by $I_f M$, and second, that because of this lower temperature and the resulting increase in the rate of $C + R$ transfer, the necessary E for equilibrium is higher than the E for an infinitely thin water film ($I_f = 0$) by the factor $\frac{I_a + I_f}{I_a}$. The extra evaporation can be thought of as producing the lower T_f.

As noted above, a similar analysis of evaporation from clothed men leads to the requisite equivalence of equations 3B, C, and D despite the fact that E will vary with the path of evaporation. Extension of the analysis permits estimation of the relative rates of evaporation required by the three possible routes of evaporation for identical skin temperatures and environmental conditions. Thus, since for equal temperature gradients from air-wall to skin ($T_s - T_a$) the right-hand members of equations 5B, C, and D are equal and differ only in the coefficient of E to preserve equality, E must increase as its coefficient decreases. Hence for the same skin temperatures and environmental conditions E will be largest when evaporation occurs initially from the skin and recondenses and re-evaporates from the clothing (smallest coefficient) (C, 5), and smallest when the evaporation occurs from the skin without subsequent condensation in the clothing (largest coefficient) (B, 5). Initial evaporation from the clothing (D, 5) requires an E intermediate between these two situations.

and skin temperatures are representative of any predictable mass of tissue remains questionable. With these uncertainties success in partial calorimetry depends largely on the degree to which negligible changes in storage are incurred. Because of the above sources of error, and those incurred in the temperature measurements themselves, useful study of the $C + R$ exchange has been restricted to the two 120°F. environments. In these environments the large $C + R$ exchange reduces the relative importance of these sources of error.

The reliability of the coefficients of $C + R$ depends not only on the thermal difference, $C + R$, but also on the accuracy of the temperature differences $T_a - T_s$ and $T_a - T_e$. Two factors enter into the reliability of the $T_a - T_{(s \text{ or } e)}$, the accuracy of the individual measurement and the reliability of the weighting formula.

The weighting procedure for an average skin temperature (T_s) is reasonably reliable inasmuch as variations in temperature of individual areas are small. In the clothed man the weighting procedure for an average surface temperature (T_e) is less reliable because of the greater temperature differences between individual areas resulting from uneven wetting and the presence of folds in the clothing. Moreover, while the emissivity of skin may be taken as unity without error, a similar assumption for clothing is not valid. The effect of a low clothing emissivity on the measurement of T_e would be to underestimate $T_a - T_e$, both where the clothing temperature is above ambient (T_e as calculated would be too low) and where clothing temperature is below ambient (T_e as calculated would then be too high). If radiation exchange only were involved, the temperature error would be self-compensating inasmuch as the error could be considered as an apparent reduction in either emissivity or radiation area. However, a real error is incurred with convection exchange, since this must be related to the true temperature. The assumed clothing emissivity of 1 here used is probably not greatly in error. Crude measurements in this laboratory gave a value between 0.85 and 0.9 for the emissivity of dry HBT. Aldrich, quoted by Wulsin (7), gives the value of 0.81 as the emissivity of HBT at low temperatures (60°C.). These values suggest a possible error in $T_a - T_e$ of 10% to 20% and a corresponding error in $\frac{C + R}{\Delta T}$. In the 120°F. environment, the measured T_e would be high by 1 to 2°C. Since water has a high emissivity at these temperatures, and since the clothing was at least partially wet in all the experiments, the error may be even smaller.

RESULTS

Nude subjects: Evaporation. Under normal circumstances the sweat-regulating mechanism adjusts sweat output to a rate adequate to maintain thermal equilibrium. As the thermal stress increases, whether external or internal (metabolic), the sweating rate progressively increases until heat dissipation by evaporation compensates for the heat gain of the body. With increasing sweat rates or with decreasing evaporative capacity of the atmosphere (high vapor pressure, low wind velocity) the sweat output eventually becomes high enough

to completely wet the surface of the subject. When that condition is reached the rate of evaporation becomes a function of two factors, wind velocity and the difference in vapor pressure between the water on the skin and in the atmosphere. However, when the wetting of the surface is not complete, then the rate of sweat output, hence sweat evaporation, is determined by the imposed thermal stress,

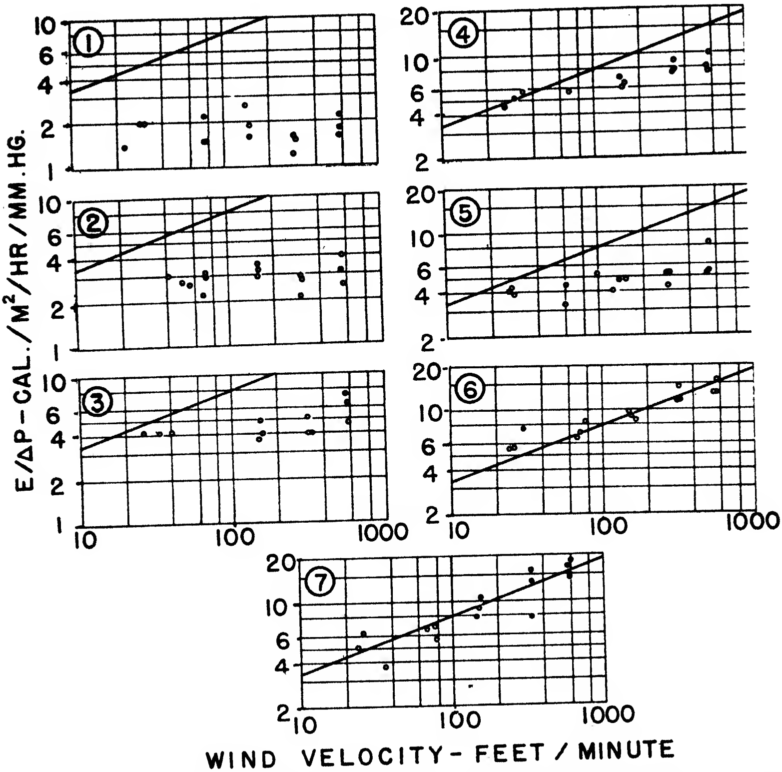


FIG. 2. EVAPORATION AS A FUNCTION OF WIND VELOCITY FOR NUDE STANDING SUBJECTS. The circled numbers correspond to the environment numbers as given in table 1. The lines are drawn according to the equation $E/\Delta P = 1.44V^{0.37}$.

and the rate of evaporation per se is independent of environmental factors. Consequently, if one is to evaluate the influence of wind velocity and vapor-pressure difference on rate of evaporation, it is necessary to confine study to those conditions where the rate of evaporation is limited by the capacity of the atmosphere to take up moisture, i.e., to the completely wetted condition.

The failure of wind velocity to influence the rate of evaporation at low sweating rates is shown in figure 2 in which the apparent coefficient of evaporation is plotted against wind velocity for the 7 environments studied. In the first two environments the rate of evaporation is independent of wind velocity. The

rate of evaporation begins to increase with wind velocity when environmental dry bulb temperature alone increases (increased sweat rate), as in environments 3 and 4, and when the evaporative capacity of the atmosphere decreases (increase in ambient vapor pressure), environment 5. Finally, with still greater reduction in the evaporative capacity of the átmosphere, environments 6 and 7, sweat is produced more rapidly than it can be evaporated, and the coefficients

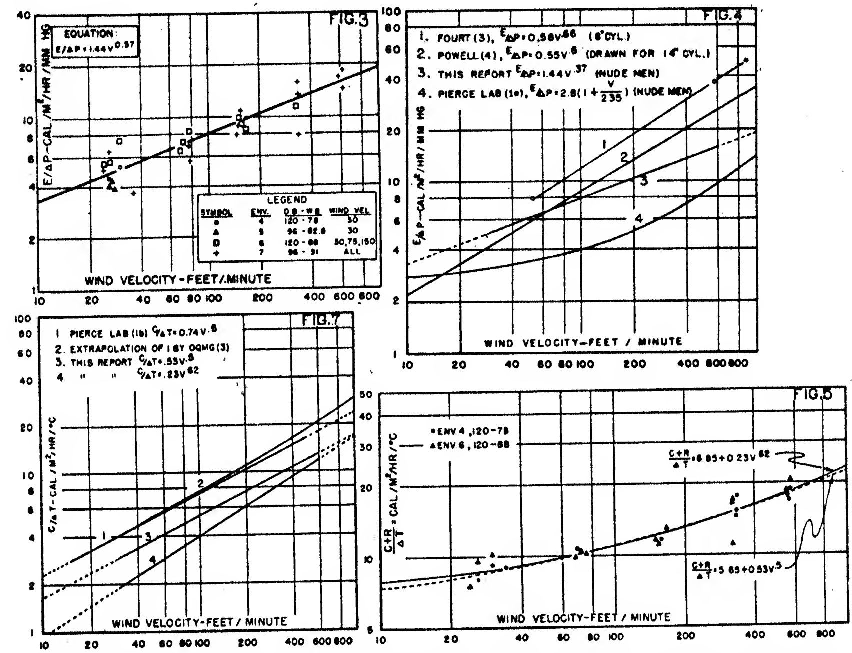


FIG. 3. EVAPORATION AS A FUNCTION OF WIND VELOCITY FOR NUDE STANDING SUBJECTS To insure complete wetting only those experiments in which 10% or more of the sweat remained unevaporated are included.

FIG. 4. COMPARISON OF VARIOUS RELATIONSHIPS THAT HAVE BEEN USED TO DESCRIBE EVAPORATION AS A FUNCTION OF WIND VELOCITY.

FIG. 5. COEFFICIENTS OF CONVECTION PLUS RADIATION PLOTTED AGAINST WIND VELOCITY. Nude standing subjects.

FIG. 7. COMPARISON OF VARIOUS RELATIONSHIPS that have been used to describe convection as a function of wind velocity.

of evaporation increase decisively with increasing wind velocity reaching limiting values indicated by the lines in figure 2.

By limiting consideration to those experiments where a high degree of wetting is present, data useful for characterizing the influence of wind velocity on evaporation can be obtained. An objective basis for selection is to include only those experiments where evaporation was less than 90% of the total sweat output, i.e., 10% or more of the sweat dripped from the man or remained on the skin. Data so selected are plotted in figure 3. These coefficients show reasonably good grouping and suggest an exponential relationship between the coefficient of

evaporation and wind velocity. A line fitted to the data by the method of least squares yields the equation $E/\Delta P = 1.44V^{0.37}$. The exponential relationship has precedent in the findings of Powell (4) on the rate of evaporation from cylinders, and has also been suggested on theoretical grounds (5). Studies of the influence of wind velocity on evaporation from completely wetted cylinders by Powell (4) and Fourt (quoted in 3) have indicated that the coefficient of evaporation varies approximately as $V^{0.6}$. In contrast, our results suggest that the coefficient is a function of $V^{0.4}$. The reasons for the difference are not clear. Two possibilities are suggested: 1) that in our experiments complete wetting of the skin was not maintained at the higher wind velocities; 2) that the human body, though often conveniently considered as consisting of a series of cylinders, differs sufficiently in its actual geometrical configuration to account for the difference.

In figure 4 the coefficients of evaporation obtained in this study are compared with those from the two studies on cylinders mentioned above and with an extrapolation used by the Pierce Laboratory (1d, e). The deviation of our results from those derived from the Pierce Laboratory equation is not surprising. This equation is an extrapolation by a questionable procedure and is based on a still air evaporation coefficient. However, the differences between our results on man and those of Powell and Fourt on cylinders will require further study and eventual explanation.

Convection and radiation. Figure 5 shows the coefficients for the combined $C + R$ calculated from the nude experiments in environments 4 and 6. The points are moderately well grouped and fall around a smooth curve. Since a certain amount of leeway is possible in fitting a curve to these points, a number of curves considered equally probable were drawn and analysed. If the assumptions are made that the convection coefficient is related to an exponential function of V and that the radiation coefficient is independent of V, the following equation is suggested:

$$\frac{C + R}{\Delta T} = (a + bV^{c})$$

where a corresponds to the radiation coefficient. Differentiation of this expression suggests that plotting of $\log \frac{C+R}{\Delta T} \Big/ \Delta V$ against $\log V$ should give a straight line having a slope equal to $(c - 1)$ and an intercept equal to $\log bc$. Establishment of b and c permits calculation of a. Values of a can be calculated for each pair of $C + R$ and V values, and the results so obtained then averaged. Alternatively $C + R$ can be plotted against the appropriate function of V, and a line fitted by the method of least squares. This procedure, which fixes both a and b, is illustrated in figure 6A for $F(V) = V^{0.5}$. Treatment of the several curves in this way leads to a series of equations whose limiting values are expressed in the two equations

$$\frac{C + R}{\Delta T} = 6.85 + 0.23V^{0.62} \tag{1}$$

and

$$\frac{C + R}{\Delta T} = 5.65 + 0.53V^{0.5}. \tag{2}$$

The equation describing convection as function of $V^{0.5}$ is tentatively favored for several reasons. The $\sqrt{V}$ relationship leads to a more acceptable value for $R/\Delta T$. The theoretical value of the coefficient, $\frac{R}{T_1 - T_2} = 4.92 \times 10^{-8}(T_1^4 - T_2^4)$ is 6.19 at the approximate temperatures of these experiments (37°C. and 48°C.). The value of 6.85 (equation 1) is thus too high even if the effective radiation area were equal to the man surface area. The coefficient of 5.65 (equation 2) gives in relation to the theoretical value of 6.19 a radiation area of 91%; this is rea-

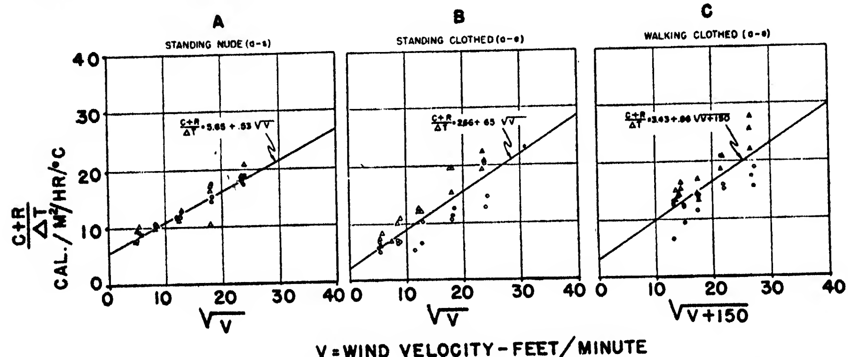


FIG. 6. CONVECTION PLUS RADIATION VERSUS WIND VELOCITY. Circles: 120°F, D. B., 78°F, W. B. Triangles: 120°F, D. B., 88°F, W. B. The coefficients are based on the temperature difference from air to skin (a − s) in the nude experiments and air to clothing (a − e) in the clothed experiments, and on actual skin surface area, not the clothed surface area.

sonably close to the estimated value of 80%. In addition, equation 1 leads to lower values for $C/\Delta T$ at very low wind velocities than does the second equation. Comparison with data available on $C/\Delta T$ at such wind velocities (1d) favors the higher convection coefficient given by the $V^{0.5}$ relationship (equation 2).

Measurements of convective exchange with cylinders for a wide range of air temperatures, cylinder sizes and wind velocities (5) have been satisfactorily correlated with air movement by means of dimensionless ratios. These correlations favor the exponent of 0.6 for V for the range of wind velocities here studied. However, until data permit a more definite choice than is now possible, the $\sqrt{V}$ relationship seems more satisfactory.

The dimensionless ratio procedure has been used to extrapolate the Pierce Laboratory data to higher wind velocities (3).[10] Lines are drawn in figure 7

[10] With this method of extrapolation the exponent of V increases with V, consequently curve 2 (fig. 7) leads to higher values than curve 1 at high wind velocities.

to represent the original Pierce Laboratory expression, the revised form as extrapolated and the two expressions suggested by the present study. The deviation of the Pierce Laboratory data from ours probably derives from the different experimental conditions employed. Their air movement was turbulent, secured by several fans in a small booth; in our studies the flow was linear.

No attempt has been made in this treatment to correct the convection coefficients for natural convection (chimney effect). It is probable that natural convection contributes significantly to the coefficients at low wind velocities; however, inadequacy both of the data and of the theoretical treatment of this problem (*see* McAdams, 5) makes such correction unprofitable at this time.

The expressions suggested here for thermal exchanges are presented only as a convenience in correlating the data and for use in interpolation. It would be foolhardy to use these equations to extrapolate beyond the conditions from which they were derived. Moreover their application to conditions where air flow is not linear may not be valid.

Clothed subjects: Evaporation. Evaporation from clothed subjects may proceed according to several different paths. In certain situations several patterns of evaporation may be occurring simultaneously at different points on the body. Three possible paths are illustrated in figure 1 B, C, and D. In an attempt to define a coefficient of evaporation, it is necessary to consider on what factors the coefficient depends. Whenever evaporation occurs from the surface of completely wet clothing, as in C or D, figure 1, the controlling factors are the same as those operative in the nude subject; namely, vapor pressure difference between surface and air and wind velocity. The situation changes, however, when as in B, figure 1, evaporation occurs from the skin and the water passes through the clothing as vapor. In this case the significant vapor-pressure difference is that from skin to air, not clothing to air. A new factor is introduced, the diffusion resistance offered to the vapor by the clothing barrier. Though wind velocity is still an influencing factor, its contribution is considerably reduced by the interposed diffusion resistance.

Though there is little reason to anticipate that the rate of evaporation from completely wetted clothing would differ significantly from the rate of evaporation from skin, several factors in the present data prevent the demonstration of this probability. After the initial warm-up period, the subject donned a fresh dry uniform and then entered the wind tunnel for the 30-minute test period. Consequently, even with the highest sweating rates, the clothing was dry during a portion of the test period. Therefore, in none of the clothed experiments was evaporation confined exclusively to the clothing surface; a portion of the evaporation must have occurred from the skin through the clothing.

The increased difficulties in assigning a mean temperature to the surface of a clothed, as compared to a nude, man have been described; these uncertainties influence the reliability of P_s(since P_s is based on T_s) and hence of $P_a - P_s$ and the evaporation coefficient, $E/\Delta P$.

An evaluation of the effective wind velocity on a man walking in a moving air

stream will be presented later. The data now to be considered have been plotted against the tunnel wind velocity.

Figures 8 and 10 indicate the effect of wind velocity on the coefficient of evaporation for standing clothed and walking clothed men in the 7 test environments.

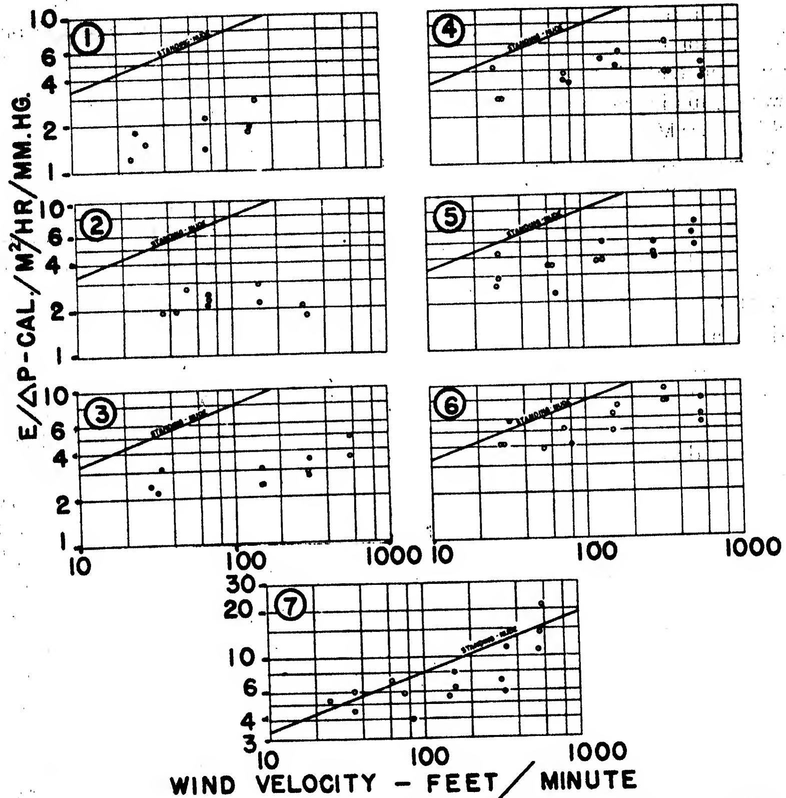


FIG. 8. EVAPORATION AS A FUNCTION OF WIND VELOCITY for clothed standing subjects. The circled numbers correspond to the environment numbers as given in table 1. The coefficients of evaporation are based on the vapor pressure gradient from clothing to air (a − e) and on the actual skin surface area of the subject, not the clothed surface area. Only those experiments are plotted in which 20% or more of the sweat remained unevaporated.

As in the nude subjects, the rate of evaporation is virtually independent of wind velocity at low sweat rates (less severe environments), but becomes progressively more dependent on wind velocity as the environmental severity increases. In the nude experiments maximal coefficients were approached when the data was restricted to those situations where less than 90% of the sweat was evaporated. For the clothed subjects (figures 8 and 10) even with a still more generous allowance for wetting (evaporation less than 80%, 20% or more unevaporated), a

progressive increase in the coefficient continues as the sweating rate increases and evaporative capacity of the environment decreases. This suggests that the allowance for wetting of the clothing is still inadequate. To test this possibility the data were separated into groups according to total sweat output (figures 9 and 11). This analysis shows a progressive increase in the coefficient of evaporation with increasing sweat rates, but there is little to suggest that maximal rates

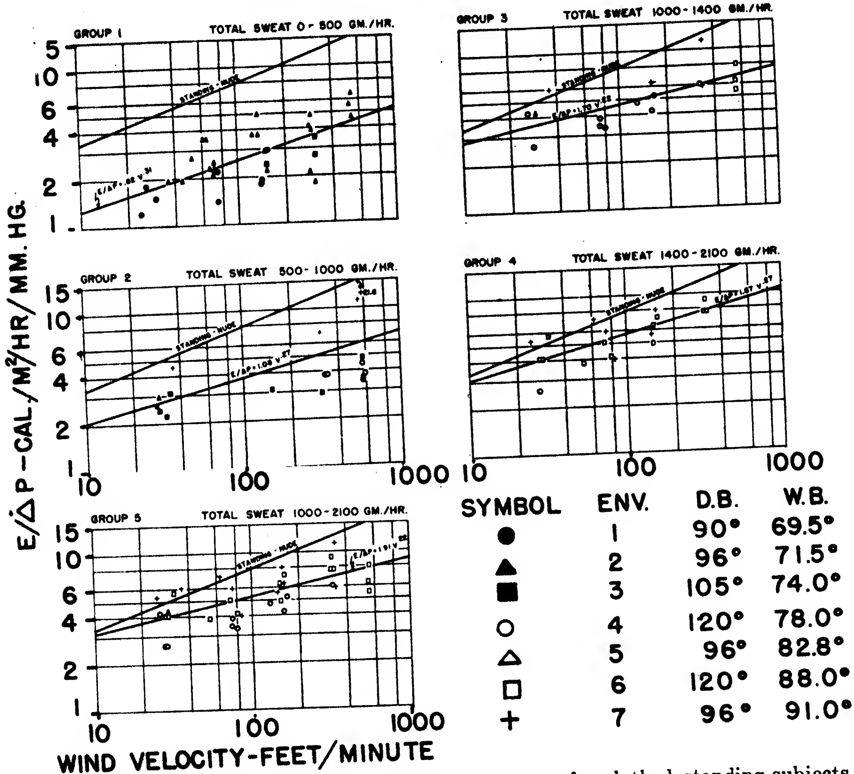


FIG. 9. EVAPORATION AS A FUNCTION OF WIND VELOCITY for clothed standing subjects. Data as in figure 8 grouped according to amount of total sweat.

are being approached, except perhaps for *group* 4, figure 11, which includes the highest sweat rates.

In a further analysis of *group* 4, the tunnel wind velocity was corrected for the increased motion of the arms and legs by adding 150 feet/minute (explained in the next section) to all wind velocities, and the coefficients were corrected to a clothed surface area, using a factor of 1.3. These corrections permit comparison of the coefficients for clothed men directly with the coefficients determined on nude subjects (fig. 12). Since most of the points fall below the values for the nude subjects, incomplete wetting of the clothed surface occurred in even the

most favorable situation, indicating that in none of the clothed experiments have maximal surface coefficients of evaporation been reached.

The 20% allowance of unevaporated sweat is probably more than adequate to ensure complete wetting of the skin. Hence it seems likely that the measured rates of evaporation under such conditions can be considered as maximal co-

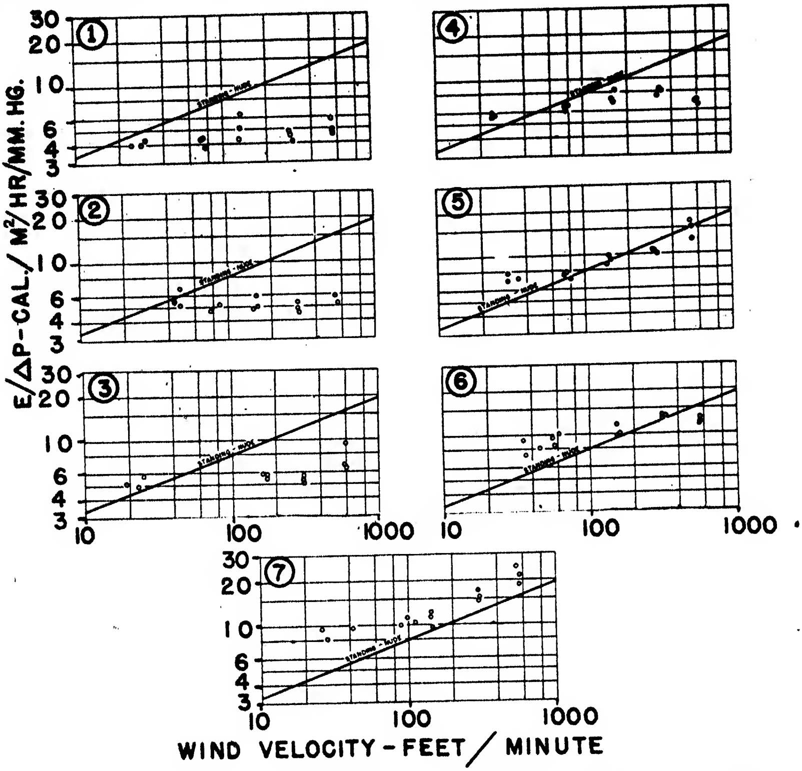


FIG. 10. EVAPORATION AS A FUNCTION OF WIND VELOCITY for clothed walking subjects. The circled numbers correspond to the environment numbers as given in table 1. The coefficients of evaporation are based on the vapor pressure gradient from clothing to air (a − e) and on the actual skin surface area of the subject, not the clothed surface area. Only those experiments are plotted in which 20% or more of the sweat remained unevaporated.

efficients, not for completely wetted clothing, but for partially wetted clothing where the evaporation occurs to varying degrees through several paths: *a*) from wet skin through the dry clothing, *b*) from the surface of wet clothing.

The situation is a complex one and is not susceptible to simple analysis or presentation in a form likely to be generally useful. The presentation given in figures 9 and 11 may be useful for some purposes. It should be noted, however, that the coefficients are calculated on the gradient from the clothing surface

to air. This is not the significant gradient for evaporation from the skin through dry clothing. Data are available on the influence of fabric porosity on the evaporation coefficient (Fourt, 3). However, they are of little help in the absence of a basis for determining the proportion of evaporation that occurs from the skin through clothing. Lacking such information, the most useful purpose of the present data is to give gross coefficients of evaporation for a range of sweat rates.

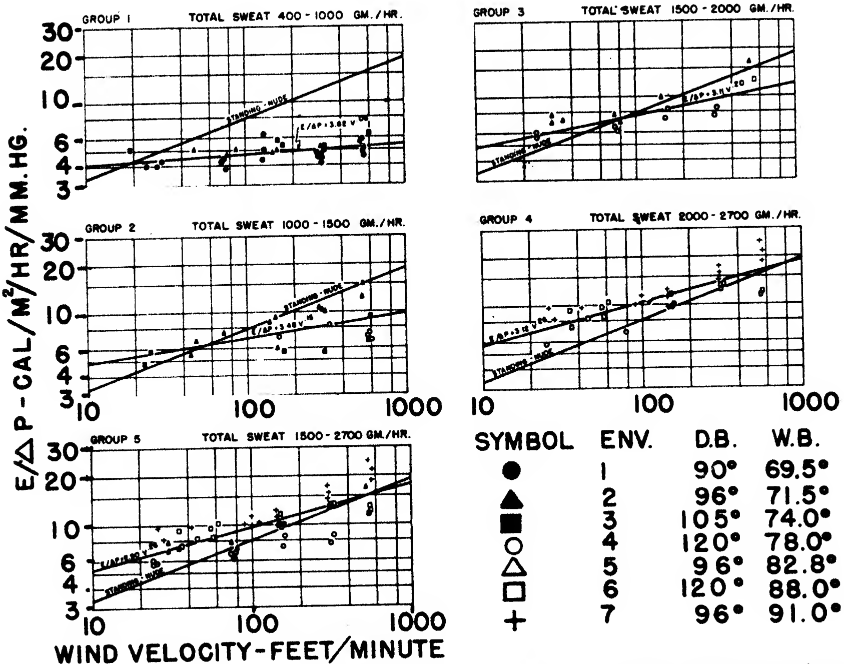


FIG. 11. EVAPORATION AS A FUNCTION OF WIND VELOCITY for clothed walking subjects. Data as in figure 10 grouped according to amount of total sweat.

Convection and radiation. The effective wind velocity increase resulting from the arm and leg motion of walking has been estimated by direct comparison of the coefficients of evaporaton and convection of standing and walking men (fig. 13). The chart shows $\frac{C + R}{\Delta T}$ for the 120°F. environments 4 and 6 and $E/\Delta P$ for those experiments where the total sweat output was between 1400 and 2100 grams/hour. The abscissal differences between the curves drawn through the points are given in table 5 and suggest that the effective wind velocity for a walking man is increased by 80 to 200 ft/min. above the measured air velocity in the tunnel. The intermediate value of 150 ft/min. is used below (also fig. 12) to correct the measured wind velocity in the walking experiments. Where this is done the symbol V' is used, where $V' = V + 150$.

The procedure used for separating C from R in the clothed experiments is based on the assumption that $C/\Delta T$ is functionally related to wind velocity in the same way in the clothed tests as in the nude ones. To this end the values of $\frac{C+R}{\Delta T}$ are plotted against $\sqrt{V}$ in figure 6 B and C. Lines fitted to the points by the method of least squares give the equations

$$\text{Standing clothed, } \frac{C+R}{\Delta T} = 2.66 + 0.65\sqrt{V}$$

$$\text{Walking clothed, } \frac{C+R}{\Delta T} = 3.43 + 0.66\sqrt{V'}.$$

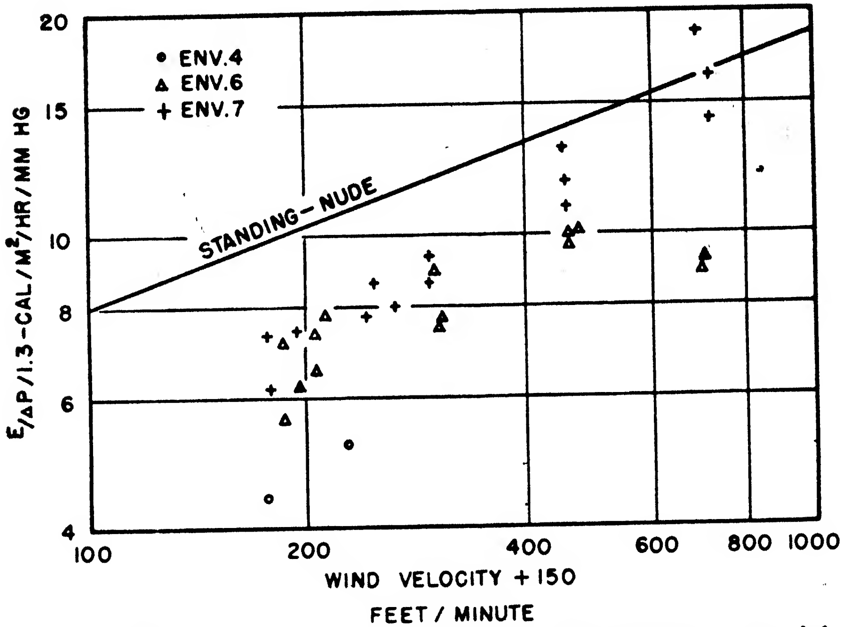


FIG. 12. COEFFICIENTS OF EVAPORATION for clothed walking subjects corrected for clothing surface area and for the apparent increase in wind velocity due to walking. Includes only those experiments in which sweat rates ranged between 2000 and 2700 grams/hr.

Consider first the coefficients of R. Since the walking man has a larger effective radiation area than the standing man, the values 2.66 and 3.43 qualitatively bear the correct relationship to each other. Quantitatively their ratio of 0.79 is somewhat lower than would have been expected on the basis of estimated radiation areas of 80% and 90% for the two conditions, respectively.

The absolute values of these radiation coefficients are much lower than anticipated and no reasonable explanation has been found for the discrepancy. Due to the larger surface area of the clothed subject, the $\frac{R}{\Delta T}$ for the clothed man

should be 20% to 30% higher than for the nude man unless the clothing emissivity is low. This possibility appears to be ruled out by the data available on the emissivity of the clothing worn.

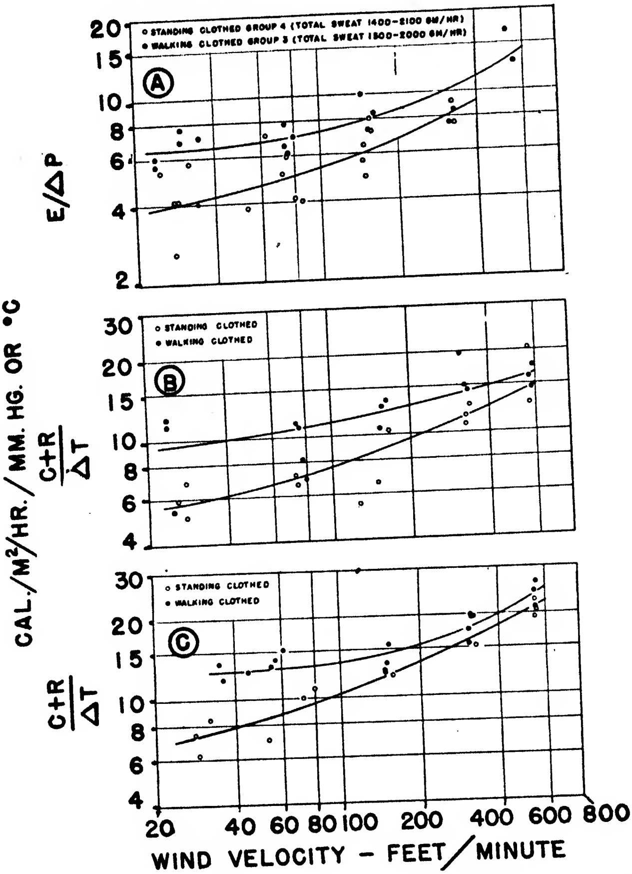


FIG. 13. ILLUSTRATION OF THE METHOD used to estimate the influence of walking on the apparent wind velocity. Smooth curves are drawn through the points from the standing clothed experiments (open circles) and walking clothed experiments (closed circles). The abscissal displacements are listed in table 5. A, presents evaporation coefficients for standing and walking experiments where the total sweat rates are in the same range. B and C show similar comparisons of convection plus radiation coefficients for environments 4 and 6, respectively.

The convection coefficients $C/\Delta T = 0.65\sqrt{V}$ and $0.66\sqrt{V'}$ are in good agreement. Their ratios to the corresponding value (0.53) for the nude subjects are 1.23 and 1.24 which are perfectly compatible with the anticipated increase in surface area of the clothed men.

Nevertheless, the total $\frac{C+R}{\Delta T}$ is lower than can be explained. The question may therefore be raised, whether the satisfactory values for $C/\Delta T$ may not be fortuitous and whether the net deficit of $C + R$ should be distributed between both the convection and radiation coefficients, making both coefficients too low. Since the possible error in measurement of $T_a - T_e$ from assuming too high a value for clothing emissivity tends to underestimate $T_a - T_e$, $\frac{C+R}{\Delta T}$ would be still lower if the correct $T_a - T_e$ were used. Thus, though $\frac{C+R}{\Delta T}$ as calcu-

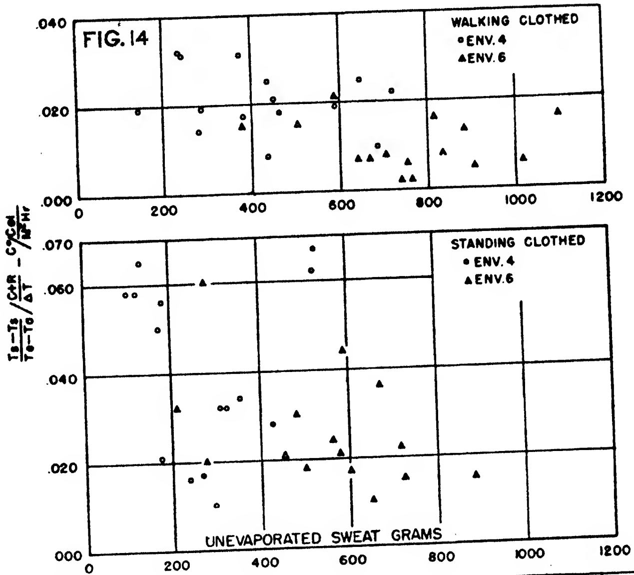
TABLE 5. *Apparent increase in wind velocity with walking*

	WIND VELOCITY ft./min.		
	Standing Clothed	Walking Clothed	Δ
	$E/\Delta P$ 1400 to 2100 gms/hr		
cals M^2 Hr			
8	300	145	155
7	215	80	135
6.5	180	40	140
	$\frac{C+R}{\Delta T}$ Env. 4		
15	610	420	190
12	360	150	210
10	225	45	180
	$\frac{C+R}{\Delta T}$ Env. 6		
22	580	480	100
15	260	180	80
13	190	50	140

lated is low, the full extent of this deficit may have been concealed by dividing by a too small ΔT.

From a practical standpoint, convection or radiation to clothed men is most satisfactorily defined in terms of the effective insulation of the clothing. However, the evidence that E can vary according to the path of evaporation independently of clothing insulation indicates that difficulty may be expected in characterizing clothing insulation from the data available. This is further shown by equations B, C and D of figure 1, where $\frac{T_s - T_e}{T_e - T_a}$ is related to the various insulation coefficients and to M and E. In the simplest situation, 6B, the clothing insulation $I_c + I_i$ is equal to $I_a \frac{T_s - T_e}{T_e - T_a}$ and in this case $I_c + I_i$ can be calculated.

When, however, evaporation deviates from this route as in C and D, $I_a \dfrac{T_s - T_e}{T_e - T_a}$ is no longer a simple function of the clothing insulation.



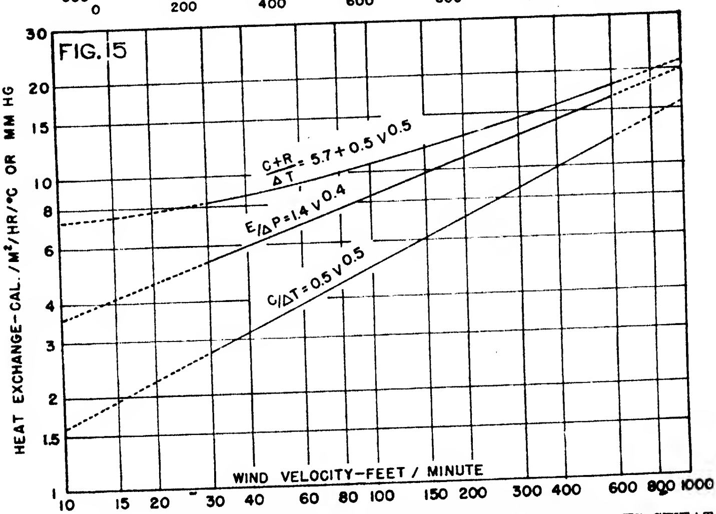


FIG. 14. APPARENT CLOTHING INSULATION IN RELATION TO UNEVAPORATED SWEAT.

FIG. 15. ROUNDED VALUES OF COEFFICIENTS OF CONVECTION, RADIATION AND EVAPORATION, NUDE SUBJECTS.

Recognizing this, but in an effort to cast some light on the effect of clothing wetness on its insulation, $I_a \dfrac{T_s - T_e}{T_e - T_a} \left(\text{or } \dfrac{1}{K_a} \dfrac{T_s - T_e}{T_e - T_a}\right)$ has been plotted against

unevaporated sweat in figure 14. The result, as anticipated, suggests that with increasing wetness of clothing its conductance increases.

Two factors suggest that the apparent insulation as plotted in figure 14 may be reasonably valid: First, in condition C (fig. 1) the value plotted, $\frac{1}{K_a}\frac{T_s - T_e}{T_e - T_a}$ should differ only slightly from the true clothing insulation, $I_i + I_c$, since the coefficient of E in the denominator of equation 6C can be expected to be very close to one. Second, condition D, fig. 1, requires that T_e be lower than T_s. In the condition here treated, where T_a is higher than T_e, a negative ratio, $\frac{T_s - T_e}{T_e - T_a}$ would result if condition D dominated. The absence of negative values in figure 14 suggests that evaporation according to path D is relatively minor.

DISCUSSION

The results of the analysis of the experiments with nude subjects appear fruitful. The coefficients of the three exchange paths studied, convection, radiation and evaporation, are all consistent with expectations and with the limited data available for comparison. The data for evaporation has no counterpart; the only reasonable comparison is with the data of Powell and Fourt (4, 3). The differences revealed by this comparison are perhaps not larger than would be anticipated, taking into account the scatter of our results and the inherent differences in the type of experiment. Further study is desirable on many grounds, but especially needed is an answer to the possible criticism that incomplete wetting was present in our experiments at the higher wind velocities.

The independent estimation of the radiation coefficients yields a satisfying confirmation of earlier work. As more information accumulates over extended ranges of conditions, the adequacy of the theoretical description of radiation exchange as applied to nude men becomes more apparent.

As with radiation, the descriptions here offered for convection exchange in nude men fall largely into the category of extension of available information to different conditions of air flow and to more severe environmental conditions. The most useful information on convection exchange at high wind velocities will probably come from study of linear air flow which occurs much more frequently at high wind velocities than does turbulent flow.

For practical use, the following rounded values are suggested for the coefficients derived from the nude experiments:

$$\text{Evaporation, } E/\Delta P = 1.4V^{0.4}$$

$$\text{Convection, } C/\Delta T = 0.5V^{0.5}$$

$$\text{Radiation + convection (120°F.), } \frac{C + R}{\Delta T} = 5.7 + 0.5V^{0.5}.$$

In these equations thermal exchange has the units Cal/M^2/hr/°C. or mm.Hg, and air velocity is expressed as feet/minute. Lines drawn from these equations are shown in figure 15.

The results from the experiments with the clothed subjects are perhaps most useful insofar as they point out the complexity of the problem and the difficulties likely to be encountered in applying the method of partial calorimetry. Most of the uncertainties of the present analysis would be eliminated if a complete heat balance were available. The evaporation coefficients require complete restudy under conditions insuring better control and greater uniformity of wetting, a difficult but very practical and important task.

The data presented here on the gross coefficients of evaporation for clothed men are of very limited usefulness, but until better information is available, they may serve to fix the order of magnitude of evaporation from partially wet clothing.

With respect to the convection and radiation coefficients from the clothed men, two alternatives are offered. The easiest course at the moment is to disregard the results on the basis of inadequate definition of the surface temperature, or measurement of storage, or both. On the other hand, if we are to accept the eminently reasonable values found for $C/\Delta T$ we are forced into the necessity of accepting what at present appears to be an unacceptable value for clothing emissivity.

SUMMARY

1. Coefficients of thermal exchange for nude men standing and for clothed men, standing and walking, have been estimated by partial calorimetry in 7 environments and at 5 wind velocities. Dry bulb temperatures ranged from 90°F. to 120°F; vapor pressures, 13 to 36 mm.Hg; wind velocities, 30 to 600 ft/minute.

2. In nude subjects the maximum coefficient of evaporation can be described by the equation $E/\Delta P = 1.4V^{0.4}$.

3. Sweating rates adequate to measure the maximum coefficients of surface evaporation in clothed men probably were not reached. Charts presenting the coefficients actually found are shown.

4. Coefficients of convection for nude men can be described by the equation $C/\Delta T = 0.5\sqrt{V}$.

5. Estimates of the convection coefficient for clothed subjects gave values 23% and 24% higher than the coefficient found for nude subjects. This is consonant with estimates of the ratio of the surface area of clothed to nude men.

6. The coefficient of radiation for nude subjects was 5.7 Cal/M^2/hr/°C. This value is in agreement with a theoretical coefficient based on emissivities of wall and skin of 1 and a radiation area equal to 91% of the surface area.

7. The coefficients of radiation for clothed subjects were much lower than would be predicted from reasonable assumptions as to emissivity of the clothing surface. No explanation of this discrepancy is offered.

8. Movement of the arms and legs while walking resulted in an increase in the apparent wind velocity. This amounts to approximately 150 ft/min. over the tunnel air flow when subjects walk at a 3 m.p.h. pace.

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